OCCURRENCE OF SOIL-DWELLING PESTS OF CABBAGE AND ONIONS IN KENYA AND MANAGEMENT OF ONION FLY USING FORTIFIED BLACK SOLDIER FLY FRASS FERTILIZER

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DECLARATION

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

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DEDICATION

I dedicate this thesis to my loving parents, Mr. Jonathan M. Onyango and Mrs. Rose N. Onyango

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LIST OF ABBREVIATIONS AND ACRONYMS

AEZ	Agro-ecological zones
ANOVA	Analysis of variance
B	Boron
BLAST	Basic Local Alignment Search Tool
BOLD	Barcode of Life Database
BSF	Black soldier fly
BSFFF	Black soldier fly frass fertilizer
C: N	Carbon: Nitrogen
CERK1	Chitin Elicitor Receptor Kinase 1 enzyme
COI	Cytochrome oxidase subunit I
CV	Coefficient of Variation
DAP	Di-ammonium phosphate
Df	Degrees of freedom
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphates
EC	Emulsifiable concentrates
EMC	Effective microorganism culture
FTIR	Fourier-transform infrared spectroscopy
GLM	Generalised linear models
GPS	Global Positioning System
ICIPE	International Centre of Insect Physiology and Ecology
ID	Identity
IPM	Integrated pest management
LH	Lower highland
LM	Lower midland
LSD	Least significant difference
NAC	N-alkyl chitosan
NBC	Benzyl chitosan
NCBI	National Centre of Biotechnology Information

N-P-K	Nitrogen-Phosphorus-Potassium
RCBD	Randomized complete block design
rDNA	Ribosomal deoxyribonucleic acid
SC	Suspension concentrates
SL	Soluble liquid
SPSS	Statistical package for the social sciences
Taq	Thermus aquaticus
UH	Upper highland
WG	Water-dispersible granule
WHO	World Health Organisation
EC	Electrical conductivity
mS/cm	Milli Siemens per centimetre

ABSTRACT

Soil-dwelling insect pests constitute a significant challenge to vegetable production and food security, with their damage being exacerbated by climate change, land degradation, and poor monitoring due to soil heterogeneity. Chitin and chitosan from sea arthropods, such as crabs, lobsters, shrimp and krill, and mass rearing of the black soldier fly (BSF) have been explored for promoting soil nutrients and crop yield. However, there is limited knowledge about the agronomic and plant health potential of BSF frass fertilizer and chitin-rich pupae exuviae in crop production. Therefore, this study determined the incidence and severity of soil-borne insect pests of onions and cabbage and their management practices in Kenya, the insecticidal potential of fortified BSF frass fertilizer on onion flies and the agronomic performance of onion grown on soils enriched with fortified BSF frass fertilizer.

A field survey was conducted to assess the occurrence, incidence and management of soil-borne insect pests of cabbage and onions in Nyandarua, Nakuru, Kiambu, Nyeri, and Kajiado counties targeting 45, 65, 32, 34, and 34 fields, respectively. Crops were randomly selected and assessed for signs and symptoms of infestation, and those affected were carefully uprooted together with the attached pests for morphological and molecular characterization. Chi square, t-test and analysis of variance (ANOVA) were performed to compare variables among counties and AEZs using SPSS. Onion flies which had the highest damage score were reared for three generations and used in contact, residual and ovicidal bioassays using the liquid fertilizer made from BSF frass fertilizer and pupae exuviae. Nevertheless, a field study was also conducted to determine the impact of amending the soil with BSF frass fertilizer and chitin-rich pupae exuviae on onion crop growth and yield. The BSF frass fertilizer was applied at a constant rate of 4.12 t ha^{-1} based on the N requirement of onion plants which is 120 kg nitrogen (N) ha^{-1}. Black soldier fly pupae exuviae was applied as a percentage of frass fertilizer based on chitin rates. For the laboratory bioassays and the field experiment, the data were subjected to ANOVA followed by mean separation using Fisher's LSD at $\alpha = 0.05$ significance level in R statistical software.

The dominant soil-borne pests of cabbage from the field survey were cabbage root fly/seed-corn fly (*Delia platura*), white grubs (*Maladera* sp.), and wireworms (*Agriotes* sp.), whereas onion fly (*Atherigona orientalis*) and sap beetle (*Urophorus humeralis*) were the major onion pests identified. Cabbage root fly had the highest occurrence of 14% and 12% in Nyandarua and

Kiambu Counties, respectively, whereas that of white grubs was 9%, 8%, and 3% in Nyandarua, Nakuru, and Kiambu Counties, respectively. Although the occurrence of onion fly was 35% in both Nakuru and Nyeri counties, a higher pest incidence of 18% was recorded in Nakuru compared to 7% in Nyeri. Sap beetles, onion flies and white grubs had the highest damage score of 4.7, 4.3, and 2.8, respectively. About 95% of farmers in the study area relied on synthetic insecticides, such as organophosphates, pyrethroids and neonicotinoids.

An increase in the level of black soldier fly pupae exuviae in the liquid fertilizer resulted in a corresponding increase in the mortality of onion fly larvae. Liquid fertilizer formulations with 20% pupae exuviae recorded the highest egg mortality of 65%, but this did not significantly vary across formulations with ≥20 pupae exuviae. The highest larval mortality of 31% was recorded for the first instar larvae of onion fly after contact exposure, while mortalities of 21% and 22%, were recorded for the second instar larvae after contact and residual exposure to liquid fertilizer formulations made from ≥20 pupae exuviae. The black soldier fly liquid fertilizer with 20% pupae exuviae reduced the overall survival until pupation by about 35% and delayed pupation by three days. In the field experiment, plots amended with BSF frass fertilizer and 8% chitin had the highest growth of seven leaves and 62 cm plant height at physiological maturity, which was higher than growth in plots amended with BSF frass fertilizer without pupae exuviae as well as those amended with commercial fertilizers. Plots amended with 8% chitin recorded the highest increase in bulb weight, bulb diameter, total onion biomass, total yield and marketable yield by 44.5%, 23.5%, 46.9% 50.5%, and 49.6%, respectively. Nonetheless, the highest total and marketable yields of 50.10 t ha^{-1} and 50.6 t ha^{-1} were recorded in plots amended with BSF frass fertilizer and 8% chitin, which was significantly higher than yields in plots amended with BSF frass fertilizer without exuviae as well as the control.

The study identified onion flies *A. orientalis* and *D. platura* as the emerging soil-dwelling pests of onions and cabbage with considerable crop damage. The findings also revealed BSF liquid fertilizer as a potential candidate for an IPM programme in controlling root flies in vegetables as a soil drench. Amending the soil with a mixture of BSF frass fertilizer and chitin-rich pupae exuviae also enhances the activity of beneficial microbes, soil nutrients and plant health, thus promoting plant growth and yield. The study recommends the utilisation of BSF frass fertilizer and pupae exuviae in enhancing crop yield and protection against soil-dwelling pests.

CHAPTER ONE: INTRODUCTION

1.1 Background to the study

Horticulture is the third largest foreign exchange earner in Kenya, contributing about 33% of the agricultural GDP and employing approximately 6 million people with over 3.5 million indirect beneficiaries (Kangai & Gwademba, 2021). Bulb onion (*Allium cepa* L.) and Cabbage (*Brassica oleracea* var. *capitata* L.) are vital vegetable crops cultivated in Kenya's low, medium and high rainfall areas, primarily by smallholder farmers for domestic and commercial purposes (Gateri *et al.*, 2018). However, vegetable farming in Kenya is significantly constrained by numerous abiotic and biotic factors such as variety, soil fertility, climate change, and pests and diseases (Ning *et al.*, 2017; Borhade *et al.*, 2018; Dianingrum *et al.*, 2019). Although numerous efforts have been put in place to develop novel cultivars with traits such as high yield, disease tolerance, early maturity, tolerance to abiotic stress, and postharvest qualities, insect pests and diseases remain the most critical challenge impacting cabbage and onion farming in Kenya (Ning *et al.*, 2017; Borhade *et al.*, 2018).

Some of the common insect pests of onion include thrips (*Thrips tabaci* Lindeman), leek moths (*Acrolepiopsis assectella* Zeller), onion flies (*D. antiqua* Meigen) and aster leafhoppers (*Macrosteles quadrilineatus* Forbes) (Diaz-Montano *et al.*, 2011; Gill *et al.*, 2015; Seto & Shelton, 2016; Alyokhin *et al.*, 2020). However, diamondback moth (*Plutella xylostella* L.), aphids (*Brevicoryne brassica* L.), cutworm (*Agrotis* spp.) and cabbage root fly/maggot (*Delia radicum* L.) have been recorded as the most devastating pests of cabbage (Reddy, 2011; Fening *et al.*, 2013). Onion flies are also emerging as major pests of onion in Kenya but there is limited information about their identity and crop loss. Indeed, soil degradation, climate change and progressive conversion of forest ecosystems into croplands are causing the proliferation of soil-dwelling insect pests, whose detrimental stages reside in the soil or within the crop, below or at ground level (Delory *et al.*, 2016; Ambele *et al.*, 2018). Consequently, about half of the onions consumed in Kenya are imported mainly from Tanzania and Ethiopia (Gateri *et al.*, 2018).

The threat of insect pests on vegetables has prompted farmers to increase the routine application of insecticides to produce high-quality products (Macharia, 2015; Marete *et al.*, 2021). However, insecticides are associated with numerous human health problems and environmental hazards

(Amoabeng *et al.*, 2017). Insecticides are noxious and responsible for frequent poisoning of agricultural workers and farmers and their toxicity has been exacerbated by excessive application, use of banned hazardous products, and the re-use of empty pesticide containers for other domestic purposes (Nyakundi *et al.*, 2012; Amoabeng *et al.*, 2017). Increased insecticide use also interferes with the natural control of insect pests, a vital ecosystem service in agriculture supported by diverse taxa, further exacerbating pest problems. Therefore, there is a need to develop sustainable pest control approaches in order to reduce the health and environmental risks associated with pesticides. Residual products obtained from the novel mass rearing of black soldier flies (*Hermetia illucens* L.), could be used in the management of pests and diseases of onion and improve the yield due to their high nutrient and chitin content.

The life cycle of the black soldier fly resembles that of other holometabolous arthropods whereby the transformation from pupae to adult stages results in pupae exuviae, which are rich in chitin and chitosan (Lagat *et al.*, 2021; Lin *et al.*, 2021). Studies have revealed that the BSF pupal exuviae have 10.18–11.85% chitin which can be used for industrial and agricultural purposes (Lagat *et al.*, 2021). Previously, aquatic invertebrates such as krills, shrimps, lobsters and crabs were utilized as primary sources of chitin (Purkayastha & Sarkar, 2020). However, the novel mass rearing of BSF has resulted in the high production of waste streams essentially made of black soldier fly frass fertilizer and chitin-rich pupae exuviae. Amending the soil with a mixture of BSF frass fertilizer and pupae exuviae has a high potential of promoting crop yield and eliciting systemic immunity by accumulating defence-related antimicrobial compounds in the plants (Hemantaranjan, 2014; Mwaheb *et al.*, 2017; Suarez-Fernandez *et al.*, 2020; Torgerson *et al.*, 2021; Wantulla *et al.*, 2022). Thus, this approach may present a cheap alternative to pesticides in managing soil-borne pests of onion while providing sufficient nutrients for optimum yield.

1.2 Statement of the problem

In 2020, Kenya imported onions worth over KES 602 million from the international market to meet the local demand, despite the vast arable land and agro-ecological conditions that are favourable for onion production. A large fraction of yield loss in Kenya is attributed to infestation and damage by pests and diseases. Soil-borne insect pests partly contributed to high onion and cabbage yield loss since they target the onion bulb and cabbage roots, which are integral for optimal yield. Onion flies contribute to a range of 20-80%, 24.6-83.7%, 50-65%, and 50-100% yield loss in the US, Poland, India, and the Netherlands, respectively (Szwejda, 1982; Finch, 1989; Loosjes, 2000; Gupta *et al.*, 2021). However, there is limited knowledge about the identity and abundance of these pests in onion and cabbage growing areas in Kenya. Therefore, there is a need to characterize the soil-dwelling insect pests affecting onion and cabbage farming in Kenya and the management practices employed by farmers.

Due to the abstruse nature of soil-dwelling pests and the associated management challenges, farmers are now applying excessive amounts of broad-spectrum insecticides such as organophosphates, neonicotinoids, diamides, pyridazinones, spinosyns and pyrethroids (Nyakundi *et al.*, 2012; Otieno *et al.*, 2015). High application of insecticides is associated with numerous adverse effects, such as damage to non-target and beneficial organisms, pest resistance, increased chemical residues in food products, and direct toxicity to farmers and agricultural workers. Although amending the soils with chitin-rich insect exoskeletons has the potential to control soil-borne pests, reduce overreliance on hazardous insecticides and enhance crop yield, there is limited information about the insecticidal activity and soil nutrient potential of BSF pupal exuviae. Numerous previous studies have assessed the industrial and agricultural application of chitin from sea arthropods such as crabs, lobsters, shrimps, and krill, which are limited in abundance. With BSF farming projected to expand drastically in Kenya and sub-Saharan Africa, there is a need to evaluate the effect of fortified BSF frass fertilizer as soil amendments on soil-borne pests of onion and crop yield.

1.3 Justification of the study

Understanding the most harmful soil-dwelling pests of onions and cabbage is essential to establishing appropriate and sustainable management approaches. Chemical pesticides threaten human and environmental health, food security, and biodiversity; therefore, replacing them with more sustainable organic soil amendments will reduce the associated health concerns, production costs, and pest damage while enhancing nature’s biodiversity. Amending the soil with fortified BSF frass fertilizer would also enhance vigorous plant growth and the yield to increase onion production to meet the local market needs (Beesigamukama *et al.*, 2020; Barragán-Fonseca *et* *al.*, 2022) . High vegetable production may also elevate Kenyan farmers' ability to compete in the global market and increase the country's foreign exchange from vegetable farming. Amending the soil with organic BSF waste streams will also enhance soil health by promoting the growth of beneficial microbiomes and improving soil structure and nutrient profile. Therefore, farmers can adopt mass-rearing of black soldier fly, which converts low-quality organic wastes into animal proteins (larvae) and create waste streams (frass fertilizer and pupae exuviae) that can replace costly commercial fertilizers and insecticides. The findings of this study lay the foundation for the utilization of fortified BSF frass fertilizer to manage insect pests of other food and industrial crops in Kenya.

1.4 Objectives of the study

The main objective was to improve the productivity of onion using chitin-enriched black soldier fly frass fertilizer for soil fertility management and pest suppression.

The specific objectives were:

i. To determine the incidence and severity of soil-borne insects of onions and cabbage and their management practices in Kenya.
ii. To assess the insecticidal activity of fortified black soldier fly frass fertilizer on onion fly (*Atherigona orientalis*).
iii. To evaluate the agronomic performance of onion grown on soils enriched with black soldier fly frass fertilizer and pupal exoskeletons/exuviae.

1.5 Hypotheses

i. The incidence, severity and management practices of soil-borne insect pests of onions and cabbage vary across different agro-ecological zones in Kenya due to differences in geographic and climatic conditions.
ii. The liquid fertilizer made from fortified black soldier fly frass fertilizer has insecticidal activity against onion flies due to high chitin quantities which favour the proliferation of cuticle-digesting chitinolytic microorganisms.
iii. Fortified black soldier fly frass fertilizer enhances the growth and yield of onion due to high nitrogen rates and controlled nutrient release.

CHAPTER TWO: LITERATURE REVIEW

2.1 Origin and botany of onion

The genus *Allium* consists of seven species commonly cultivated for economic purposes: the bulb onion (*Allium cepa* L.), shallot (*A. cepa* var. *ascalonicum* L.), leek (*A. ampeloprasum* var. *porrum* L.), garlic (*A. sativum* L.), Chinese chive (*A. tuberosum* L.), potato onion (*A. cepa* var. *aggregatum* L.), and the Japanese bunching onion (*A. fistulosum* L); however, there are more than 100 *Allium* species (Havey, 1995). *Allium* belongs to the Amaryllidaceae family and the Allioideae subfamily (Bremer *et al.*, 2009). Bulb onions are the most valuable *Allium*, with total global production of approximately 101.52 million tonnes in 2020 (FAO, 2022). Habitats of the member *Allium* are widely distributed in North America, North Africa, and the Eurasian continent. Reference to onion, leek, and garlic as food and medicine can be traced back to biblical accounts of the Israelites' exodus from Egypt during 1500 BC. The use of onion and garlic as food can also be traced back to the sixth century BC in India. The Romanian and Greeks authors, such as Hippocrates, Theophrastus, and Pliny, also described onions as early as 430 BC, 322 BC, and AD 79, respectively (Havey, 1995). Therefore the bulb onions seem to have originated from Central Asia, but other literature traces onion farming back to Iran and Egypt (Shultz, 2010).

Allium cepa consists of plants with multiple bulbs, such as tree onions and shallots, and single-bulb onions, also called globe onions. Bulb onions are biennial plants with fibrous and adventitious roots with three to eight glaucous leaves (Marrelli *et al.*, 2019). The crop is grown for its edible bulb, which forms from concentric, enlarged leaf bases. Onions are grown from the plant's tiny black seeds sown directly to the field or into the nursery before transplanting. Bulbs vary in colour, shape, and pungency, but warmer conditions produce sweeter flavour than other climatic conditions. The crop's pungency is attributed to the high composition of volatile sulphur compounds produced from the hydrolysis of *S*-alk(en)yl cysteine sulphoxide precursors by the alliinase enzyme (Havey, 1995). Onion plant height and size of mature bulbs vary depending on abiotic and biotic factors, but the shape can be elongated, ovoid, or globose based on the cultivar (Lim, 2016). The outer leaves dry to form the thin protective coat, whereas the inner leaves thicken as the crop grows.

2.2 Onion and cabbage farming in Kenya

Onion is one of the oldest vegetables cultivated over 5000 years ago (Pareek *et al.*, 2018). The crop is valued due to its pungent bulb, which is rich in vitamins B6 and C, potassium, phosphorus, magnesium, and calcium (Pareek *et al.*, 2018). Onion is used as a spice in curries and salads, as a condiment, or cooked with other vegetables. Onions are also valued for their medicinal properties, such as anti-oxidation, antithrombotic, anti-cholesterol, anti-inflammation, anti-diabetic, and antimicrobial (Lanzotti, 2006; Pareek *et al.*, 2018). The crop is a rich source of dietary flavonoids with three vital phytochemicals: organosulphur, fructans, and flavonoid compounds (Pareek *et al.*, 2018). The organosulphur and flavonol compounds are responsible for the anti-oxidant qualities of onion, while the steroidal saponins inhibit the absorption of cholesterols. Flavonoids also prevent cancer risks through mechanisms such as inhibition of heat shock protein, regulation of p53 protein, tyrosine inhibition kinase, cell cycle arrest, and blockage of Ras protein expression (Duthie *et al.*, 2000; Pareek *et al.*, 2018).

Onion is among the most consumed vegetables in Kenya (Gateri *et al.*, 2018; Birithia *et al.*, 2021). Bulb onion farming in Kenya primarily targets the domestic market, whose demand surpasses the local supply, resulting in importation from neighbouring countries such as Ethiopia and Tanzania (Horticultural Crops Directorate, 2020; Birithia *et al.*, 2021). In 2020, the total area under onion production in Kenya was 6,992 ha resulting in a total production of 115,113 tons with a total value of KES 4.87 billion (Horticultural Crops Directorate, 2020) (Appendix 1). The crop is primarily grown in high-altitude areas (Mai Mahiu and Naivasha in Nakuru County), mid-altitude zones (Kieni in Nyeri County), and low-altitude zones (Emali in Makueni County and Kimana and Mbirikana in Kajiado County) for local consumption (Birithia *et al.*, 2021) (Appendix 1). Bulb onions grown in Kenya include the red onion varieties (Red pinoy, Bombay red, Red tropicana, Jambar F1, Red passion, Red creole) and white onion varieties (Texas grano). Despite an increase in onion farming in Kenya (14.3 t ha^{-1}), production remains low, compared to lead global producers such as Korea (63.5 t ha^{-1}), USA (54.4 t ha^{-1}), Australia (54.2 t ha^{-1}) and Spain (54.1 t ha^{-1}) (FAOSTAT, 2016). Kenya's low onion yield can be attributed to numerous factors, such as low-yielding varieties, poor handling practices, low quality seeds, climate change, pests and diseases (Gathambiri *et al.*, 2021; Kiura *et al.*, 2021). Indeed, pests and diseases contribute the most to onion yield loss in Kenya and other sub-Saharan countries.

Cabbage, whose origin is the South and Western Coast of Europe, ancient Greek and the Eastern Mediterranean, is broadly cultivated in Kenya as a commercial and subsistence crop (Maggioni *et al.*, 2010). In Kenya, cabbage is primarily grown in highlands with an elevation range of 800-2900 m a.s.l, especially in counties such as Nyandarua, Nakuru, Kiambu, Nyeri, Kericho, Bungoma, Bomet, Meru, Kisii, Narok, Murangam Trans-Nzoia, Laikipia and Elgeyo Marakwet Counties (Macharia *et al.*, 2005; Muriuki *et al.*, 2002; Horticultural Crops Directorate, 2020). Different varieties of cabbage have been developed to suit the different agroclimatic conditions of Kenya. The common cabbage varieties in Kenya include Pruktor F1, Gloria F1, Victoria F1, Queen F1, Rosy F1, Baraka F1, Green Challenger F1, Riana F1, Copenhagen, Zawadi F1, Green Coronet F1, Santa F1, Super Master F1, Fiona F1 and Globe Master F1 (Daniel & Muindi, 2023). Cabbage is primarily consumed due to its rich source of Vitamins A, C and K, proteins, lipids and carbohydrates, as well as minerals such as calcium, phosphorus, zinc, potassium and iron, which are integral for human health (Singh *et al.*, 2010; Daniel & Muindi, 2023). Cabbage is also a good source of health-promoting phenolics, glucosinolates and antioxidants. Nevertheless, cabbage is a rich source of iodine which is central for the normal functioning of the brain, nervous system and thyroid gland (Daniel & Muindi, 2023). Cabbage farming serves as a vital source of employment for the Kenyan population, significantly influencing food and national security.

2.2.1 Constraints to cabbage and onion production

Despite the high demand for cabbage and onion in Kenya, their production is constrained by numerous biotic and abiotic factors. Draught is the most critical abiotic stress impacting onion farming in lower midland agro-ecological zones, such as Oloitokitok in Kajiado and Emali in Makueni (Kichamu *et al.*, 2018). These zones are prone to high temperatures and extreme droughts, prompting farmers to use irrigation, which increases the production costs. Previous studies have revealed up to 53% yield loss due to drought stress, especially when it occurs at the beginning of the bulb formation stage in onion and head formation in cabbage (Hanci & Cebeci, 2015). Under drought conditions, plants experience restricted moisture uptake, which affects physio-biochemical processes resulting in low yield (Junaid *et al.*, 2021). Soil salinity also affects onion farming in Kenya, especially under basin irrigation schemes. High concentrations of salts, such as Cl – and Na +, can affect the physiological functioning of crops and growth when taken up by the plant (Chaudhry *et al.*, 2021). It also interferes with effective water absorption causing oxidative stress and may result in approximately 51% yield loss (Golldack *et al.*, 2014; Hanci & Cebeci, 2015). Cold stress may also disrupt the physiological activities of bulb vegetables, especially at the seedling stage, and may increase the incidence of dumping off and root rot diseases (Choi *et al.*, 2022). Abiotic stress interrupts physiological, morphological, and biochemical functioning, but the extent of damage depends on the variety of onions.

Although there has been a remarkable improvement in the breeding of cabbage and onion varieties that can withstand abiotic stress, many biotic factors continue to constrain onion farming. Indeed, biotic stress caused by pests, diseases, and weeds causes significant constraints to vegetable farming, accounting for over 80% yield loss. Damping off caused by *Pythium* sp., *Phytophthora* sp., *Rhizoctonia solani*, and *Fusarium* sp., and purple blotch caused by *Alternaria porri*, have been identified as the most prevalent onion diseases in Kenya, with 29.5% and 38.1% incidences, respectively (Makelo, 2004). Purple blotch infestation can result in about 50% yield loss in the absence of efficient management practices (Manjunathagowda *et al.*, 2022). Iris yellow spot disease (IYSD) has recently been detected in Kenya, with an incidence range of 70-82% in Emali, Kimana, Mbirikana, and Kieni. Basal rots caused by *Fusarium* spp. also account for a significant reduction in onion yield in major growing zones of Kenya (Currah *et al.*, 2012). Other common onion diseases include Downy mildew, rust, white rot, and black mould (Currah *et al.*, 2012). The most devastating fungal diseases of cabbages include the Downy Mildew (Perenospora parasitica), Yellows or Fusarium Wilt (*Fusarium oxysporum* f. sp *conglutinans*), Blight (*Alternaria brassiciola*), Leaf Spot (*Alternaria brassicae*), Black Leg (*Phoma lingum*), Damping off (*Pythium debaryanum*) and Sclerotinia rot (*Sclerotinia sclerotiorum*). Cabbage is also affected by bacterial diseases such as the Clubroot of cabbage (*Plasmodiophora brassicae*), Bacterial Soft Rot (*Pectobacterium carotovorum*), Wire Stem (*Rhizoctonia solani*) and Black Rot (*Xanthomonas campestris* pv. *campestris*) (Arim *et al.*, 2019; Daniel & Muindi, 2023). Apart from diseases, insect pest constitutes the most critical constraint to onion farming in Kenya.

Among the common insect pests of onion include thrips (*Thrip tabaci*), onion flies (*Delia antiqua*), onion leaf miner (*Liriomyza sativae*), and cut worm (*Agrotis* spp.) (Delahaut & Newenhouse, 2003; Birithia *et al.*, 2014; Haile *et al.*, 2016; CABI, 2022b). According to Birithia *et al.* (2011), thrips cause the highest crop damage due to their ability to transmit Iris yellow spot disease caused by a virus in the family *Bunyaviridae* and genus *Tospovirus*. Onions are more susceptible to injury by thrips during the bulb enlargement stage resulting in a significant reduction in the final yield. Feeding by thrips also causes the silvering of leaf tissues and destroys the aesthetic nature of the bulbs (Shiberu & Mahammed, 2014). Other than thrips, onion fly larvae cause significant crop damage under high infestation. The commonly studied species of onion fly is the *Delia antiqua* (Meigen); however, other polyphagous species, such as the *Delia platura* (Meigen), *Delia florilega* (Zetterstedt), and *Atherigona orientalis* (Schiner) have also been associated with a wide range of crop damage in some countries (Gupta *et al.*, 1991; Ellis & Scatcherd, 2007). Nevertheless, insect pests constitute the most critical drawback to cabbage production in Kenya. The key pests infesting cabbages in Kenya include the diamondback moth (*Plutella xylostella*), cabbage aphid (*Brevycoryne brassicae*), cabbage webworm (*Helula undalis*), cutworm (*Agrotis* spp) and cabbage root fly/maggot (*Delia radicum*) (Blackshaw *et al.*, 2012; Savage *et al.*, 2016). The initial generation of *Delia* Spp. causes substantial crop damage by feeding on the developing epicotyl and roots of onion and cabbage seedlings causing plant death and reducing crop stand. However, the second and third generation of onion fly feeds on the expanding bulbs causing bulb rot, which opens routes for infection by other opportunistic pathogens (Wilson *et al.*, 2015).

Kenya has a stable market for cabbage and onion, but the local production does not meet the market demand, especially for onion. According to Muendo & Tschirley (2004), high production costs are due to weeding, irrigation, seed costs, and pest management costs, affecting the crop's profitability. Previous studies in Pakistan have reported between 71 and 76% reduction in onion marketable yield due to weed competition (Khokhar *et al.*, 2006). In a survey by Gitonga *et al.*, (2006), weed control was perceived as the third most critical challenge facing onion farming in Kenya, after diseases and pest management. Despite the high production costs, buyers can also collude to buy vegetables from local farmers at lower prices, further lowering the crops' value to farmers (Waijanjo *et al.*, 2009). With the recent increase in the costs of pesticides, insect pests and diseases continue to be the critical challenge constraining onion farming in Kenya. Soil-borne pests, in particular, pose a critical challenge due to their cryptic feeding habits.

2.2.2 Soil-borne pests of onions and cabbage

Soils are typically heterogeneous in qualities such as temperature, moisture and soil texture, which affects the distribution of soil-dwelling pests leading to negative binomial clumped distributions. Such aggregations frequently impede the detection and quantification of soil-borne pests using conventional sampling methods. Root herbivory has been reported in insects, molluscs, nematodes and rodents; however, plant/nematode interactions are the most documented (Ester & Huiting, 2007; Hill, 2008; Ali *et al.*, 2015; Johnson & Rasmann, 2015; Shukla *et al.*, 2016; Nguetti *et al.*, 2018; Eves-van den Akker, 2021). Six out of the twenty-six orders of insects are well-documented as below-ground herbivores of plants (Brown & Gange, 1990; Van Der Putten, 2003). Among these, the larval stages of endopterygotes such as Lepidoptera, Diptera and Coleoptera, primarily feed below ground, whereas exopterygotes (Orthoptera, Isoptera and Hemiptera) are classified as both subterranean and above-ground herbivores (Brown & Gange, 1990; Gossner *et al.*, 2015; Savage *et al.*, 2016; Nyamwasa *et al.*, 2017; Ambele *et al.*, 2018). Although it is difficult to monitor the movement of soil-dwelling pests, they likely engage in more than "trivial movements" primarily to search for new food sources. Large herbivores, such as the *Maladera* spp. and *Agriotes* spp. (Figure 2.1), burrow through the soil to greater depths in search of food and escape unfavourable conditions, while small root herbivores, such as root flies, whose early instars make the invasion, only use the existing cavities in the soil (Capinera, 2008). Root flies/maggots (Anthomyiidae) are undoubtedly the most devastating root-feeding dipterans.

The damage level caused by root flies/maggots larvae varies based on the stage of growth of the host plant (Figure 2.1). For instance, the pest causes symptoms similar to water deficit when the host is between 10 to 30 days old after transplanting causing symptoms such as delayed growth, poor quality inflorescence and lodging (Meraz-álvarez *ets al.*, 2020). At seedling stage root maggots are typically found at the base of the plant, with their herbivory limited to the crown causing substantial damage to the main stem and root system. In some cases, galleries caused by larval feeding can be observed on the main stem; orifices also appear on the stem through which the third instar larva exits the crop to undergo soil pupation (Meraz-álvarez *et al.*, 2020). Pupae are generally seen at the site where the infected plant has been extracted and can also be detected adhering to the root substrate. While young plants are significantly tolerant, crops older than 30 days can withstand the damage and thrive without showing symptoms. Nonetheless, the affected plants will be smaller with poor quality, especially when the larvae superficially feed on the main stem's external tissues penetrating the basal leaves, which turn yellow and wilt (Dunne & Coffey, 2020). Cabbage crops may also show a bluish tinge as if suffering from nutrient deficiency (Figure 2.1).

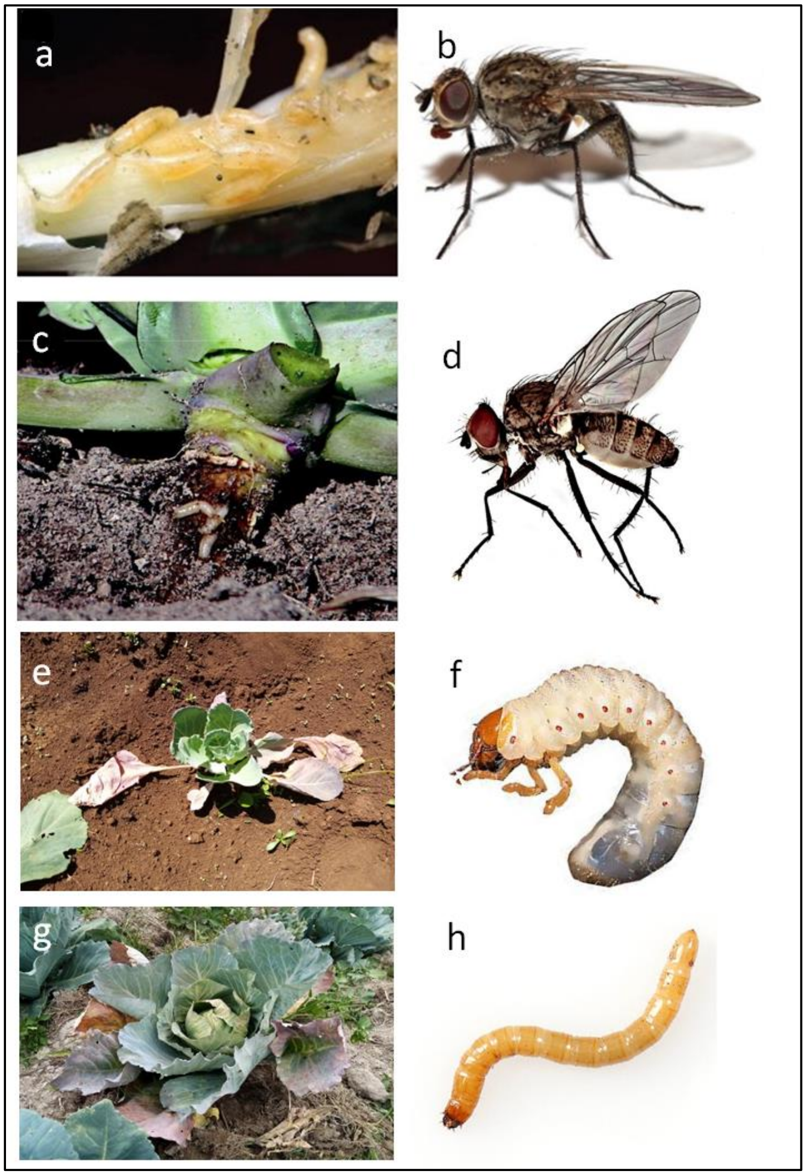

Figure 2.1: Some of the most common soil-borne pests of onion and cabbage: onion maggots feeding on the below ground stem of onions (a), onion fly (b), cabbage affected by white grub root herbivory (c), white grub (d), cabbage root crown affected by cabbage maggots (e), cabbage root fly (f), cabbage affected by root herbivory by wireworms (g), and wireworms (h) (Etzler, 2013; GoldenHarvest, 2023; Meraz-álvarez *et al.*, 2020; Tejeda-Reyes *et al.*, 2023)

2.2.3 Onion flies and their damage

The four species of root flies commonly associated with *Allium* spp. include *D. antiqua*, *D. florilega*, *D. platura* and *A. orientalis* (Savage *et al.*, 2016; Collier *et al.*, 2020; Mlynarek & Sim, 2021). Major hosts in this category include bulb onions, shallot, Welsh onion, leek, garlic and chives. Damage intensity by onion fly varies with crop stage, host variety and cropping season. In New York, which is the top producer of onions in the US, severe damage has been recorded between early and mid-summer, primarily due to *Delia platura* (Capinera, 2008). Whereas, in North America's great Lake region, *D. antiqua* larvae cause the most substantial crop damage by directly foraging on young plants early in the season (McDonald *et al.*, 2004). Gravid female flies oviposit at the base of the onion plant causing infestation at the root zone and subterranean feeding (Moretti *et al.*, 2021). The highest rate of infestation has been observed between mid- and late July in Pennsylvania, US (Moretti *et al.*, 2021), September in Egypt (Ragheb & Abu-shall, 2021) and July/August in Pakistan (Wagan, 2014). In contrast, in the UK, the first generation emerges in May/June (Agriculture and Horticulture Development Board, 2022).

In the northern temperate zones, root flies undergo three generations annually during the growing season and overwinter as pupae in the soil before eclosing in spring to begin infestation (Vernon *et al.*, 1987). The first generation of the onion fly infests the host at the seedling stage causing the most substantial crop damage. Damage by first-generation onion fly ranges from 20-80% in the US (Nault *et al.*, 2006; Moretti & Nault, 2020), 50-65% in India (Gupta *et al.*, 2021) and 24.6-83.7% in Poland (Szwejda, 1982). The second generation infests onion fields at the vegetative stage and causes considerably lower damage ranging from 1.2-5.2% (Szwejda, 1982). Although mature bulbs are more resistant to onion fly infestation, mechanical damage during harvesting can cause an infestation in the field leading to post-harvest yield loss (Capinera, 2008). For instance, a previous study in New York, US, found 8.6-26.0% damage by *D. antiqua* on onions which had been undercut using power-driven commercial lifters (Eckenrode & Nyrop, 1986). Wounds created by onion fly also act as pathways for infection by soil-borne fungus and bacteria. Although onion fly larvae damage acts as a pathway for bacterial infection, the puparium may also harbour the bacterium enabling its survival during harsh conditions (McDonald *et al.*, 2002; Hoepting *et al.*, 2004; Capinera, 2008). Nevertheless, gravid female onion flies are attracted to exudates from rotting onions for oviposition (Hoepting *et al.*, 2004). However, further interrogation is required to assess the interaction between onion fly larvae and pathogen infection.

Although *D. platura* and *A. orientalis* have been reported in major onion-growing areas in Kenya, there is limited information about their incidence and damage potential. *Delia platura* Meigen (Diptera: Anthomyiidae) is undoubtedly the world's most widespread polyphagous anthomyiid with substantial abundance in vegetable gardens and field crops (Darvas & Szappanos, 2003; Saumure *et al.*, 2006; Michelsen & Baez, 2010; CABI, 2022b). *Atherigona orientalis* (Schiner) is a polyphagous pest that feeds on plant materials damaged by other pests, but it can also be a primary pest in solanaceous vegetables and onions. For instance, *A. orientalis* is a major pest of *Capsicum annuum* in Nigeria, causing severe damage to both ripe and unripe pepper fruits, whereas in Korea, the pest has been reported on cabbage, cauliflower, tomatoes and garlic (Ogbalu *et al.*, 2005; Suh & Kwon, 2016). *Atherigona orientalis* is regarded as a minor pest of onions in the United States and India, but under heavy infestation, damaged young plants develop flaccid morphology with droopy leaves which is often aggregated in the field due to clustered oviposition and movement of larvae to adjacent healthy plants (Hoshizaki *et al.*, 2020). One onion fly larva can destroy several seedlings but mature onions are significantly tolerant to the pest. However, mechanical bulb injury and secondary infection by soil-borne pathogens may exacerbate the infestation of mature bulb onions (Capinera, 2008; Nault *et al.*, 2011).

2.2.4 Biology of onion fly (*Atherigona orientalis*)

Atherigona orientalis Schiner (Diptera: Muscidae) thrives under a wide range of climatic conditions and completes a life cycle in 24-68 days (Herawani *et al.*, 2019). Eggs are white and cylindrical, measuring 0.20 mm wide and 0.82 mm long. Parallel ridges run across the egg's dorsal surface and converge posteriorly, whereas the anterior end remains tightly fitted with a thin lid (Srivastava & Pandey, 1968). Eggs are laid in clumps at the exact location, and more than one female fly can oviposit at the same spot. *Atherigona orientalis* lays 21-191 eggs during a life span, which hatch within 1-2 days. The hatched larva undergoes three instars which can be differentiated based on the cephalopharyngeal skeleton (Santolamazza-Carbone *et al.*, 2017). The first and second instar larvae are tiny and un-described, whereas the third instar larvae can reach 7.78 mm long and 0.95 wide (Grzywacz & Pape, 2014). The pupae are dark orange to dark red, measuring 4.03 mm long and 1.62 mm wide, and lasting 6-8 days (Herawani *et al.*, 2019). *Atherigona orientalis* adults have a yellow abdomen and grey thorax with a body length of about 4 mm. The larvae forage on a wide range of materials and can cause severe damage to both unripe and ripe vegetables (Ogbalu *et al.*, 2005).

2.2.5 Management of onion fly

Management of onion pests primarily entails the use of chemical pesticides. Carbolic acids and bisulphide of carbon were the primary control approach of root flies as early as the 19th century (Cook, 1881). In the early 20th century, onion farmers switched to chlorides of mercury which were used as seed coats, seedbed drenches or furrow dust. However, the discovery of organochlorines in the 1940s revolutionised the management of onion fly with the adoption of chlordane and hexachloride insecticide as post-emergence drenches and dusts. With the development of insecticides such as heptachlor, dieldrin and aldrin, many insect pests including the cabbage root fly were effectively controlled (Davis & Mcewen, 1965). However, in the mid-20th century incidences of insect resistance to DDT were first reported on *Musca domestica* in Denmark and Sweden–1946, as well as in *Aedes sollicitans* (United States) and *Culex pipiens* (Italy)–1947, among many other insects (Metcalf, 1989). Resistance of root flies against organochlorine insecticides became more widespread in the 1960s, especially in Canada and the United States (Read, 1965). Organochlorines were also associated with critical environmental and human health risks and were later banned from use in many countries worldwide, prompting farmers to switch to organophosphates.

Presently, the management of onion flies principally involves the application of organophosphate insecticides such as diazinon and chlorpyrifos (Joseph & Zarate, 2015). Chlorpyrifos is favoured because it is relatively insoluble in water, hence less susceptible to leaching. However, frequent application of heavy doses of organophosphates has resulted in chemical residues in food, soil and water bodies causing human and environmental health concerns (Hunt *et al.,* 2003). As such, the usage of organophosphates is restricted in many countries globally. Neonicotinoid, pyrethroid, diamide, ryanodine receptor activator, spinosyn, and pyridazinone insecticides have been introduced as a feasible alternative to organophosphates with over 75% larval mortality

(Joseph & Zarate, 2015). These insecticides are primarily applied as soil drenches but can also be applied by coating the seeds or dipping onion seedlings before transplanting. For instance, film-coating seeds with chlorpyrifos reduce the rate of insecticides required to protect onion seedlings from destruction by onion fly larvae (Jyoti *et al.*, 2003). Dipping seedlings into imidacloprid may also reduce larval infestation by 67% (Bažok *et al.*, 2012).

However, many pesticides are non-selective and can destroy non-target organisms such as predators, pollinators, and nitrogen-fixing microorganisms (Sánchez-Bayo, 2012; Stanley & Preetha, 2016). Additionally, the efficacy of insecticides reduces with time due to the development of resistance by the target pests (Després *et al.*, 2007). Applying chemical pesticides and inorganic fertilizers also exposes farmers to chemical poisoning, and can leach into the soil, poisoning the soil fauna and the nearby water bodies (Osoro *et al.*, 2016). Due to these challenges, there is an increasing demand to design eco-friendly methods of controlling crop pests and diseases. One of the potential approaches is to use black soldier fly frass fertilizer and chitin-rich pupae exuviae to control both soil-borne and above-ground pests.

Studies show that adding chitin to the soil induces repellent root exudates and favours the population of chitinolytic microorganisms that digests the polysaccharide chitin to disaccharide chitobiose, thus degrading the eggshells of nematodes and onion flies (Chen & Peng, 2019; Wantulla *et al.*, 2022). Black soldier fly pupae shells are considered the best alternative to chemical pesticides in controlling insect pests and diseases due to the abundance of chitin, an amino polysaccharide polymer with pesticidal properties (Younes *et al.*, 2012). The shells are also rich in nitrogen, thus, are a vital source of soil nutrients. Although, numerous studies have focused on the extraction of chitin from sea arthropods such as crustaceans, recent studies show that black soldier fly pupae are a better source of chitin, which can be extracted through chemical treatments and superheated water hydrolysis process, or direct incorporation of the pupae exuviae into the soil (Younes *et al.*, 2012; El Knidri *et al.*, 2016; Bhavsar *et al.*, 2021).

2.3 The biology of black soldier fly

The adult black soldier fly (BSF) (*Hermetia illucens* Linnaeus) is wasp-like insect bearing distinctive white and black colour patterns over its body. The adult fly may resemble other *Hermetia* spp., requiring peer evaluation by a taxonomist before commercialisation (Surendra *et* *al.*, 2020). The life cycle of the black soldier fly resembles that of other holometabolous arthropods, whereby the adult fly lacks feeding mouthparts and can live for about two weeks surviving on water. The life cycle of BSF is influenced by environmental factors, such as light intensity, humidity and temperature (Liu *et al.*, 2017). The adult fly is 13–20 mm long and possesses a single pair of well-developed wings, two long antennae, and three pairs of legs. Adult males are smaller than females and possess an aedeagus and a pair of hooks that facilitate mating while the females have a retractile tubular oviduct on the last abdominal segment that can be used to tell the genders apart (Caruso *et al.*, 2014). The BSF is a eurygamous insect and requires a wide rearing space to facilitate its nuptial flight. The females lay between 400-1000 eggs that are usually deposited in cracks or crevices near decaying substrates (Caruso *et al.*, 2014). The eggs are ovoid about 1 mm long taking 3.5 days and 4 days at 30˚C and 27-29 ˚C, respectively, to hatch (Caruso *et al.*, 2014).

Once the larvae emerge, they feed on the surrounding organic substrate and grow through six successive larval stages. The growth rate of the larvae depends on the availability of food and the temperature; the optimum temperature for larval growth is 30˚C (Chia *et al.*, 2018). The larvae are photophobic and saprophagous feeding on decomposing organic matter. They have strong feeding mouthparts that also aid in their locomotion. As shown by Caruso *et al.* (2014), the larval body of BSF comprises of 11 segments covered with bristles and hairs and can reach up to 6 mm in width and 20mm in length. The first five stages appear almost similar except for the body size. However, the sixth instar (pre-pupae) which is brown ceases to feed and withdraw from the substrate and transforms into pupae which are motionless with rigidified cuticles measuring 12-25 mm long and rich in calcium salts (Caruso *et al.*, 2014). It takes about two weeks for the adult to emerge after the pre-pupae stage. The emergence of an imago leaves behind a pupal shell that is dry, rigid and rich in chitin.

2.4 Black soldier fly pupal exuviae as sources of chitin

Black soldier fly has gained global attention due to its ability to convert organic wastes into proteins, acting as a cheap but nutrient-rich source of livestock feeds (Makkar *et al.*, 2014; Chia *et al.*, 2021). Mass rearing of the insect also results in the production of waste streams comprising substrate wastes which can be further composted to yield frass fertilizer and pupae exuviae that is rich in chitin. According to Caruso *et al.* (2014), the pupal exuviae makes up about 10% of the total dry weight of the pupae. About 20% of the BSF exuviae is chitin, while ash (calcite; CaCO3) and lipid account for 10% and 7%, respectively, at 8% moisture content (Bhavsar *et al.*, 2021). Although, levels of chitin in BSF pupae exuviae have been reported to be as high as 23% (Lin *et al.*, 2021), some studies have recorded as low as 10.18% (Lagat *et al.*, 2021) and 14.1% (Smets *et al.*, 2020) chitin rates. This difference could be attributed to different methods of chitin extraction used in different studies. Nonetheless, BSF pupae have the highest rate of chitin compared to other development stages such as larvae, pre-pupae and adult, which have 3.6%, 3.1%, and 2.9% chitin rates, respectively (Bhavsar *et al.*, 2021). The α-chitin is the most common form of chitin in several insects as detected by Fourier-transform infrared spectroscopy (FTIR) at bands near 1650, 1620, and 1550 cm^{-1} (Waśko *et al.*, 2016; Smets *et al.*, 2020; Lin *et al.*, 2021). Fourier-transform infrared spectroscopy is also used to characterize chitin and chitosan from other sources including krill, shrimps, lobsters and crabs and fungi. The level of chitin and chitosan in BSF pupa exuviae is higher than in sea arthropod shells which have been predominantly used to produce these compounds (Tolesa *et al.*, 2019; Bhavsar *et al.*, 2021). For instance, chitin yield from shrimp shells at 110 ˚C based on ammonium-based ionic liquids (ILs) ranges from 10.0% to 14.7%, while that of crab shells is about 12.6% (Setoguchi *et al.*, 2012; Tolesa *et al.*, 2019).

2.5 Overview of chitin and chitosan

Chitin is a linear polymer of β(1-4) linked N-acetyl-D-glucosamine and D-glucosamine and is the second most abundant natural carbohydrate polymer after cellulose; it is also the most common aminopolysaccharide polymer (Lin *et al.*, 2021). Chitin is often considered a derivative of cellulose, but it possesses acetamide groups at the C-2 position, which differentiates it from the latter (Dutta *et al.*, 2004). An acetamide group replaces the hydroxyl group at C-2 in chitin with a chemical structure (C8H13O5N)*n* (Lin *et al.*, 2021). Similarly, chitosan is a derivative of chitin formed through N-deacetylation of chitin to a varied extent characterized by the degree of deacetylation. Chitosan (α (1→4)-linked 2-amino-2-deoxyβ-D-glucopyranose) is a copolymer of glucosamine and N-acetylglucosamine (Dutta *et al.*, 2004). Chitin is a homopolymer of acetamido-2-deoxy-bD-glucopyranose, but some of the glucopyranose residues exist in a deacetylated form as 2-amino-2-deoxy-b-D-glucopyranose (Zargar *et al.*, 2015). Nonetheless, chitin exists in association with other proteins, polysaccharides and minerals.

Chitosan structure is characterized by a high composition of D-hexosamine, which is highly distributed along the polymeric chain. The prevalence of a free amino group in the chitosan chain is responsible for its ability to disintegrate in dilute aqueous acid solvents making it a salt in solution (Aranaz *et al.*, 2012). Both chitin and chitosan are renewable polymers that are naturally occurring with features such as biocompatibility, adsorption, non-toxicity, and biodegradability. Unlike cellulose, chitosan has a versatile reaction due to the prevailing NH_2 groups (Younes & Rinaudo, 2015).

Chitin occurs naturally in the shells of arthropods and must be extracted through demineralization, deproteinization and decolouration processes (Tolesa *et al.*, 2019). The first step of extracting chitin from BSF pupal exuviae is the removal of minerals (demineralization) by acidification using either CH_3COOH, HCOOH, HNO_3, H_2SO_4, or HCl (Barber *et al.*, 2013; Lopes *et al.*, 2018; Tolesa *et al.*, 2019). Since $CaCO_3$ is the most abundant mineral in the shells of arthropods, HCl is preferred in dissolving the mineral at temperatures lower than 100 ˚C (Lopez-Moya *et al.*, 2019). However, HCl is highly aggressive and can cause a hydrolytic effect on chitin structure lowering the molecular weight (Tolesa *et al.*, 2019). The mineral-free products can be deproteinised using alkaline solutions or through enzymatic processes (Abdou *et al.*, 2008; Tolesa *et al.*, 2019). Enzymatic deproteinization can be achieved using *Bacillus mojavensis*, *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus metschnikovii,* but it is time-consuming and yields low-quality products compared to chemical treatment (Younes *et al.*, 2012; Tolesa *et al.*, 2019). The chemical treatment approach involves dissolving the product of demineralisation in aqueous solutions of NaOH at 65-100 ˚C, followed by purification using organic solvents (Younes *et al.*, 2012; El Knidri *et al.*, 2016). After demineralisation and deproteinization, astaxanthin is extracted in a depigmentation process to obtain a colourless product (Aranaz *et al.*, 2012). Chitosan can be derived from chitin by removing the acetyl group using 50% NaOH solution at 100 ˚C (El Knidri *et al.*, 2016).

Due to the environmental risks associated with the conventional methods of chitin and chitosan extraction, eco-friendly approaches such as biological degradation using *Bacillus* *lichenformis* A6 (Caligiani *et al.*, 2018; Lin *et al.*, 2021), microwave irradiation (El Knidri *et al.*, 2016; Pintowantoro *et al.*, 2021) and superheated water hydrolysis (Bhavsar *et al.*, 2021) have been developed. For instance, Lagat *et al.* (2021) reported 11.85% chitin in BSF pupae exuviae after using protease-producing bacteria such as *Bacillus subtilis* and *Pseudomonas aeruginosa* for extraction. According to Lagat *et al.* (2021), biological extraction methods are cheap, eco-friendly and yield high-quality chitin compared to chemical methods.

2.6 Application of chitin in crop production

Chitin and chitosan have a broad application in agriculture, the medical and industrial food processing sectors, among many other sectors (Elieh-Ali-Komi & Hamblin, 2016; Wang *et al.*, 2020). In Agriculture, chitin and chitosan are essentially applied in crop health and nutrition, biological pest control, soil conditioning and anti-transpiration (Shamshina *et al.*, 2020). Chitin enhances crops' natural defence against pests and is a vital plant anti-stress agent, growth stimulant and elicitor of secondary metabolite production (Hassan & Chang, 2017; Orzali *et al.*, 2017; Shamshina *et al.*, 2020). Due to their insecticidal properties, chitin has the potential to solve the persistent challenges associated with chemical pesticides in crop production. Chemical pesticides are harmful to the environment, expensive, prone to pest resistance, exhibit high phytotoxicity, and are toxic to non-target living organisms, including livestock (Baibakova *et al.*, 2019; Hasanuzzaman *et al.*, 2020).

2.6.1 Chitin as fertilizer

Chitin has gained attention as a source of fertilizer due to its ability to biodegrade in the soil releasing ammonia which promotes crop growth and enhances the development of beneficial microorganisms (Dahiya *et al.*, 2006; Shamshina *et al.*, 2020). According to Andronopoulou & Vorgias (2004), chitin biodegradation in the soil is achieved by bacterial chitinases, including β-N-acetylhexosaminidases, exochitinases and endochitinases. Endochitinases catalyse the hydrolysis of bonds that exist along the polymer, producing oligomers which are further degraded by exochitinase releasing diacetylchitobiose units at the polymer ends. Beta-N-acetylhexosaminidases then produce N-acetylglucosamine monomers from the oligomers to yield ammonia as the end product (Velásquez & Pirela, 2016; Shamshina *et al.*, 2020). The decomposition of chitin is also influenced by chitinolytic bacteria such as actinomycetes 5A and 8A and *Flavobacterium* species (Shamshina *et al.*, 2020).

Conventionally, farmers have been amending the soil with shrimp meal to supply crops with sufficient nutrients for optimum growth and yield. A field trial by Aklog *et al.* (2016) compared the growth of hydroponically grown tomatoes treated with shrimp meal fertilizer against those treated with a commercial fertilizer called HYPONeX. Tomatoes treated with HYPONeX had the highest growth and yield performance compared to those treated with shrimp meal fertilizer. The low performance of shrimp meal was attributed to the presence of calcium carbonate that inhibited timely decomposition and nutrient release (Shamshina *et al.*, 2020). To overcome this challenge, numerous studies have suggested that insect exoskeletons should be fermented using chitinolytic bacteria such as actinomycetes 5A and 8A, *Flavobacterium* species or symbiotic lactic acid bacteria before planting to enhance the breakdown of chitin into nutrient forms that can be utilised by plants (Manucharova *et al.*, 2006; Duan *et al.*, 2011; Shamshina *et al.*, 2020; Setiawan *et al.*, 2021)

2.6.2 Effect of chitosan on agronomic performance of crops

Amending the soil with black soldier fly frass fertilizer and chitin-rich pupae exuviae has been associated with high crop growth and yield (Beesigamukama *et al.*, 2020; Barragán-Fonseca *et al.*, 2022; Wantulla *et al.*, 2022). Spiegel *et al.* (1988) and Xue *et al.* (2018) reported a high growth rate in wheat and Chinese cabbage after enriching the soil with chitin. According to Shahrajabian *et al.* (2021), enriching the soil with chitosan enhances plant photosynthetic activities and optimises tolerance against abiotic stress. Foliar application of chitin at early maize stages has been associated with improved plant height, leaf area, leaf number, total dry mass and crop yield (Mondal *et al.*, 2013). Nevertheless, chitin application may also enhance phenol content and anti-oxidation properties (Pirbalouti *et al.*, 2017). In an experimental study by Xu & Mou (2018), the application of chitosan increased lettuce leaf growth, shoot biomass, chlorophyll index, leaf electron transport rate, maximum photochemical efficiency and photochemical yield. Nonetheless, enriching the soil with chitin remediates polluted soils and improves the soil quality by lowering the level of anionic and cationic heavy metals (Hataf *et al.*, 2018). Chitin and chitosan can also act as biostimulants in crops by inducing root growth and enhancing nutrient uptake and phytohormone production (Shahrajabian *et al.*, 2021). As a biostimulant, chitin has the added advantage of enhancing tolerance to abiotic stress, increasing photosynthetic activities and eliciting the expression of defensive genes (Pichyangkura & Chadchawan, 2015; Petropoulos, 2020).

2.6.3 Chitin and chitosan as elicitors of plant response against pests

Numerous studies have evaluated the impact of chitin on the defence of plants against insect pests and diseases. According to Shamshina *et al.* (2020), chitin and chitosan enhance the resistance of plants against pests by altering tissue metabolism and activating defence genes. Plants have specific receptors that recognize high molecular weight chitin and initiate a defence response through phytoalexin production (Shimizu *et al.*, 2010). The enzymes that trigger phytoalexin formation typically exist in an inactive state and only get activated once plant cells encounter an elicitor. For example, in cruciferous crops, Chitin Elicitor Receptor Kinase 1 enzyme (CERK1) has been identified as the primary receptor of chitin oligosaccharides (Shamshina *et al.*, 2020).

Enriching the soil with chitin and chitosan has been associated with a reduction in the incidence of bacterial wilt of tomatoes by 30.3% and 25.0%, respectively (Kemboi *et al.*, 2022). This can be attributed to the direct antibacterial activity of chitosan through cell lysis (Kumaraswamy *et al.*, 2018). According to Malerba & Cerana (2019), chitin and chitosan can also induce host resistance against bacterial infection through a hypersensitive reaction around the damaged cells, which causes programmed cell death and inhibit the further spread of the disease. According to Kumaraswamy *et al.* (2018), the lower incidence of bacterial wilt in tomatoes may also be attributed to the production of enzymes such as superoxide dismutase peroxides, chitinases and catalases that mediate metabolic pathways in plant defence responses.

Chitin and chitosan also exhibit insecticidal properties against insect pests from different families. For instance, Rabea *et al.* (2005) evaluated the insecticidal activity of chitosan derivatives (N-alkyl chitosan (NAC) and benzyl chitosan (NBC)) against the third instar larvae of *Spodoptera littoralis* and reported 100% larval mortality in treatments with NBC. Rabea *et al.* (2005) also observed irregularities in the ecdysis process suggesting that chitosan derivatives interfere with the ecdysone hormone. Similar results were reported by Zhang *et al.* (2003) on

Helicoverpa armigera, *Plutella xylostella*, *Rhopalosiphum padi*, *Sitobion avenae*, *Metopolophium dirhodum*, *Myzus persicae*, *Hyalopterus prun*, and *Aphis gossypii,* when the insects were directly exposed to chitin derivatives. Chitin and chitosan may also elicit secondary metabolites such as alkaloids, terpenes, phenols, and flavonoids in plants which can inhibit the infestation by herbivorous insects (Salimgandomi & Shabrangi, 2016; Fernández *et al.*, 2020). The secondary metabolites may repel insect pests or attract natural predators, hence offering eco-friendly management of crop pests (Orlita *et al.*, 2008; Dweba *et al.*, 2017; Lopez-Moya *et al.*, 2019; Wantulla *et al.*, 2022). However, further research is required in this area to interrogate the interaction between chitin and herbivorous pest infestation.

CHAPTER THREE
OCCURRENCE AND MANAGEMENT OF SOIL-DWELLING INSECT PESTS OF ONIONS AND CABBAGE

3.1 Abstract

Soil-borne pests pose a significant challenge to vegetable farming in Kenya, contributing to yield loss and food insecurity. A field survey was conducted to determine the occurrence, incidence, and practices adopted by farmers in the management of soil-dwelling insect pests of cabbage and onions in Nyandarua, Nakuru, Kiambu, Nyeri, and Kajiado counties. Fifty plants were randomly selected from each field and used to measure the incidence and severity of the damage by soil-borne pests on a scale of 0-5. Infested plants were uprooted intact with the soil and transported to ICIPE for pest identification. Findings from 210 fields revealed that *Delia platura*, *Maladera* sp., *Agriotes* sp. *Atherigona orientalis* and *Urophorus humerali* are the key soil-borne pests of cabbage and bulb onion. The occurrence of *Atherigona orientalis* and *Urophorus humeralis* in onion fields was 35% and 14.7%, respectively in Nakuru and Nyeri Counties. In cabbage fields, *D. platura* had the highest occurrence of 14.0% and 12.3% in Nyandarua and Kiambu Counties, respectively. The occurrence of *Maladera* sp. was 9.3%, 6.7%, and 3.2% in Nyandarua, Nakuru, and Kiambu Counties, which was comparable to *Agriotes* sp.: 9.4%, 4.7%, and 2.2%, respectively. *Delia platura*, *A. orientalis* and *U. humeralis* had the highest incidence and damage severity in cabbage and onion fields. Over 95% of farmers applied synthetic insecticides, such as alpha-cypermethrin, profenofos and cypermethrin, lambda-cyhalothrin, and imidacloprid and beta-cyfluthrin, for pest management. The study demonstrates that there is a significant threat of soil-dwelling pests on vegetable production in Kenya despite the frequent use of broad-spectrum insecticides. Soil-borne pests have diversified their hosts as in the case of *A. orientalis* and *U. humeralis* resulting in significant crop damage. Therefore, effective, sustainable, and affordable management strategies are required.

Key words: Soil-borne pests, occurrence, incidence, pest management, *Brassica oleracea* L. and *Allium cepa* L.

3.2 Introduction

Cabbage and onions are among the most commonly grown vegetable crops in Kenya for dietary and economic purposes (Horticultural Crops Directorate, 2020). However, the yields of the two crops have declined significantly due to pests and disease infestation. The challenge has worsened due to climate change, soil degradation, and the progressive conversion of the forest ecosystem to croplands, among other factors (Seif & Nyambo, 2013; Ambele *et al.*, 2018; Amfo & Baba Ali, 2021). Soil-borne pests, whose detrimental stages are found in the soil or within the crop, below or at ground level constitute a considerable fraction of vegetable pests (Delory *et al.*, 2016; Ambele *et al.*, 2018). Management of soil-dwelling insects has been difficult due to their abstruse nature such as nocturnal feeding. Additionally, they can deploy numerous defence mechanisms, ranging from the release of pathogen alarm behaviour to mutual grooming habits to get rid of conidia from the cuticles (Yanagawa *et al.*, 2008; Hussain *et al.*, 2010).

Although soil-borne insect pests are significantly detrimental to vegetable farming, they are generally poorly documented in SSA. This can be attributed to their cryptic feeding habits, which complicate scouting and monitoring practices (Nyamwasa *et al.*, 2018). Monitoring of soil-borne insect pests is also complicated by other factors associated with their life cycle. For instance, species in the Scarabaeidae family (scarabs or beetles) complete their life cycle in approximately one year (Hill, 2008; Alyokhin *et al.*, 2020), while the termite life cycle varies from 3 to 8 years, during which they cause critical crop damage of up to 100% (Hill, 2008; Nyeko *et al.*, 2010). Furthermore, soil degradation, habitat destruction and climate change have increased the proliferation of soil-borne pests. Therefore, the objective of this study was to determine the occurrence, incidence and severity of soil-borne insect pests of cabbage and onion crops in various agro-ecological zones of Kenya to generate vital information for the development of appropriate management approaches.

3.3 Materials and Methods

3.3.1 Description of the study area and sampling procedure

A field survey was carried out in Nyandarua, Nakuru, Kiambu, Kajiado and Nyeri Counties to determine the prevalence, incidence and severity of soil-borne pests of cabbage and onions in the main growing zones of Kenya (Figure 3.1). The counties were chosen to reflect the major growing regions of cabbage and onions in Kenya's high-altitude (Nyandarua, Nakuru and Kiambu counties) and middle-altitude zones (Nyeri and Kajiado counties) (Table 3.1). The counties experience different agro-climatic conditions such as precipitation, altitude and temperature as shown in Table 3.1 below. The numbers of surveyed fields per county were Nyandarua (45), Nakuru (65), Kiambu (32), Kajiado (34) and Nyeri (34), thus totalling to 210 fields. Fields in each county were chosen with the help of local agricultural extension officers, according to the availability of target crops. Fields assessed were located along major and feeder roads at a regular interval of 5 km. Sample size determination per county and field was as recommended by Sseruwagi *et al.* (2004). Nevertheless, a questionnaire was used to assess farmers' details, cropping system, pest management approach and frequency of insecticide application (Appendix II).

3.3.2 Assessment of pest incidence and damage severity

A zigzag pattern was used in each field and crop sampling was done along the walking path targeting plants both inside the field and near the edges. In each field, fifty plants were randomly selected along the path and assessed for infestation symptoms such as wilting, stunting, discolouration of leaves and stems, loss of vitality and tissue distortion. Those exhibiting these symptoms were uprooted and examined for infestation signs, such as the presence of actively foraging larvae, and the profusion of insect frass, eggs and pupae near the crown. Percentage pest incidence was determined by dividing the number of infested plants by the total number of observed plants as shown in the formula below.

$$Pest\ incidence\ (\%) = \frac{\text{Number of infested plants}}{\text{Total plants observed}} \times 100$$

Five distinct categories (score scale) were developed to evaluate the severity of soil-borne pest infestation on each plant. A score of 1 indicated absence of damage or infestation, a score of 2 indicated mild infestation (5% of the plant parts damaged), a score of 3 indicated average infestation (>5 and <50% of the plant parts damaged), a score of 4 indicated significant infestation (>50% of the plant parts destroyed), and a score of 5 indicated extremely high infestation levels, severe damage and stunting or dead plants (Ogecha *et al.*, 2019).

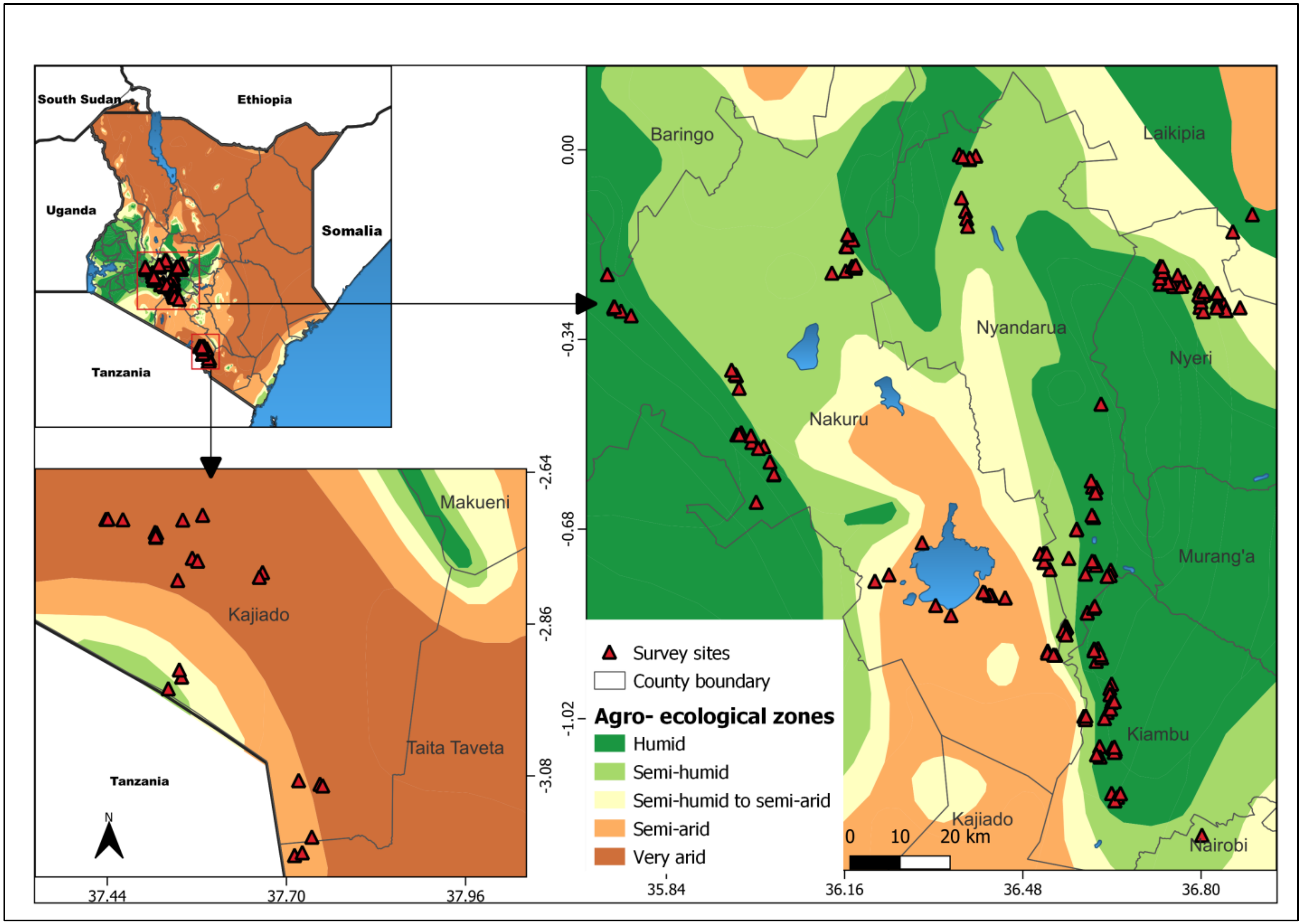

Figure 3.1: An illustration of the five study areas (counties) in humid, semi-humid, semi-arid and very arid agro-ecological zones of Kenya

Table 3.1: Characteristics of the counties covered in the survey of soil-borne insect pests

County	Sub-county	Agroecological Zones AEZ	Target crop	Altitude (m.a.s.l)	Mean Temperature (˚C)	Mean annual rainfall (mm)
Nakuru	Molo, Bahati & Njoro	Upper highland (UH1)	Cabbage	2400-3000	10.0-14.6	1150-1600
	Molo South,	Upper highland (UH2)	Cabbage	2400-3000	10.0-14.6	1000-1200
	Naivasha & Njoro	Lower highland (LH3)	Cabbage & Onion	2250-2280	15.0-15.2	800-900
	Naivasha	Lower highland (LH4)	Onion	2190-2280	15.0-15.6	800-900
Kiambu	Lari & Kijabe	Upper highland (UH1)	Cabbage	2400-3000	10.0-14.6	1150-1600
	Kijabe	Upper highland (UH2)	Cabbage	2400-3000	10.0-14.6	1000-1200
Nyandarua	South Kinangop	Upper highland (UH1)	Cabbage	2400-3000	10.0-14.6	1150-1600
	North Kinangop	Upper highland (UH2)	Cabbage	2400-3000	10.0-14.6	1000-1200
	Ol-Kalou & Ol Joro Orok	Upper highland (UH3)	Cabbage	2370-2430	13.7-14.7	900-1100
Nyeri	Kieni	Upper highland (UH3)	Onion	2370-2430	13.7-14.7	900-1100
Kajiado	Oloitokitok	Lower midland (LM5)	Onion	970-1390	20.1-22.7	420-520

Source: (Jaetzold & Schmidt, 1983)

3.3.3 Sample collection

Plants showing signs and symptoms of infestation by soil-dwelling pests were extracted intact with the soil adhering to the roots, placed in four-litre containers and labelled with GPS coordinates, date of collection and host name (Meraz-álvarez *et al.*, 2020). The size of collected samples per field ranged from 1 to 20 based on pest incidence. Collected samples were kept in rearing chambers until adult emergence for classification and laboratory behavioural assays.

3.3.4 Morphological characterization of soil-borne insects associated with cabbage and onions

Larvae of the click beetles were morphologically distinguished by examining the following set of characteristics defined by Glen and colleagues (1943). The body is straight, with nine abdominal segments visible dorsally, the ninth segment terminating in a blunt point, and the tenth segment lying ventrad to the ninth with or without hooks; three pairs of well-developed, sub-equal thoracic legs; a lyre-shaped frontoclypeal, labrum fused with the anterior margin of the frons and clypeus to form a rigid nasale; labium and maxillae fused; and biforous spiracles.

Larvae of the scarab beetles were identified using the morphological characteristic key by Šípek & Ahrens (2011). The body is 15.5-22 mm in length; smooth yellow cranium width 1.85-2.6 mm. The apex and preciliae of the mandible are brown to black, with a white preclypeus; lyriform frontal sutures; a subtrapezoidal clypeus with a slightly shorter anterior margin than posterior margin; and a weakly sclerotized preclypeus. Labrum with two pairs of prominent setae in the posterior half, trilobed anteriorly, and lateral lobes subdivided into mini lobes. Antennomere with a large sensory spot and two smaller ventral spots, as well as a small spot, bearing minute setae at the apex; raster teges covering at least the distal half of the ventral surface of the last abdominal segment.

Adult sap beetles were identified using the morphological characteristic key by Gillogly (1962). The body elongates, with at least three exposed chitinised dorsal segments; bilobed labrum with a tooth on the inner side behind the apex; ligula with wide laterally protruding paraglossae; stout palpi with a truncate and thickened terminal segment; transverse mentum, emerging in front; larger scutellum. The antennae are short, compact, and round in outline; the prosternal process behind the coxae is wide, round and depressed, reaching the mesosternum; and the prothorax is narrower than the elytra.

Onion flies were identified using the keys and illustrations by Grzywacz & Pape (2014). Palpi and antennae dark brown to black; pale grey pruinose thorax with a pair of sublateral black spots on the 3rd, 4th, and 5th abdominal tergites, about 3.8 mm in length, bare arista; coarse reticulation on the ventral surface. Molecular characterization was done to further confirm the species of the collected insect.

Morphological keys by Darvas & Szappanos (2003) and Savage *et al.* (2016) were used to identify cabbage root flies. Greyish body, 5-9 mm long, moderately setose without body or wing colouration; A1 vein extends to wing margin; upper calypter smaller or larger than lower one; arista's longest hair is shorter than the first flagellomere width; bare propleuron; hind tibia's apical posteroventral seta is almost similar to the adjacent setulae or absent. Molecular characterization was done to further confirm the species of the cabbage root flies.

3.3.5 Molecular characterization

3.3.5.1 Sampling, DNA extraction and amplification

Insect pests from onion and cabbages were collected, preserved in absolute ethanol then brought to the *icipe* Arthropod Pathology Unit for processing, whereby genomic DNA was extracted from individual insects using the Isolate II Genomic DNA Kit (Bioline, Meridian Bioscience, London, United Kingdom), following the manufacturer's instructions. The resultant DNA was eluted in a final 50 µL volume then quality and quantity checks were done using the Nanodrop 2000/2000c Spectrophotometer (Thermo Fischer Scientific, Wilmington, USA). For characterisation, the mitochondrial COI gene region was targeted using LepF1 5' ATTCAACCAATCATAAAGATATTGG 3' and LepR1 5' TAAACTTCTGGATGTCCAAAAAATCA 3' markers (Hajibabaei *et al.*, 2006) in addition to amplification of the Domain 2 (D2) region of 28S large subunit rDNA using LepD2 Fw 5' AGTCGTGTTGCTTGATAGTGCAG 3' and LepD2 Rev 5' TTGGTCCGTGTTTCAAGACGGG 3' markers (Campbell *et al.*, 1994; Goolsby *et al.*, 2006). The PCRs were carried out in a total reaction volume of 20 µL containing 5X My *Taq* Reaction Buffer (5 mM dNTPs, 15 mM $MgCl_2$, stabilizers and enhancers), 0.5 pmol μl^{-1} of each primer, 0.5 mM $MgCl_2$, 0.0625 U μl^{-1} My *Taq* DNA polymerase (Bioline) and 15 ng μl^{-1} of DNA template. These reactions were set up in the Eppendorf Mastercycler® Nexus Gradient Thermal Cycler (Eppendorf, Hamburg, Germany). The following cycling conditions were used: initial denaturation for 2 min at 95 °C, followed by 40 cycles of 30 sec at 95 °C, 30 sec annealing (52 °C for LepF1/R1 and 58.8 °C for LepD2 Fw/Rev) and 1 min at 72 °C, then a final elongation step of 10 min at 72 °C. The amplicons were resolved through a 1.2% agarose gel, and then bands on the gel were visualized and documented using the KETA GL imaging system trans-illuminator (Wealtec Corp, Meadowvale Way Sparks, Nevada, USA) (Figures 3.7 & 3.8). Thereafter, the bands were excised and purified using Isolate II PCR and Gel Kit (Bioline) following the manufacturer's instructions then shipped to Macrogen Europe BV (Meibergreef, Amsterdam, the Netherlands), for bi-directional sequencing.

3.3.5.2 Sequence analyses

The sample sequences were assembled and edited using Geneious Version 8 (http://www.geneious.com) (Kearse *et al.*, 2012). The primer sequences were identified and removed from the consensus sequences generated (from both the forward and reverse reads). For conclusive identification of the species from both markers, similarity searches were conducted by querying the consensus sequences via BLASTn (Basic Local Alignment Search

Tool) algorithm at the GenBank database hosted by the National Centre of Biotechnology Information (NCBI). This algorithm aligns and compares the queried consensus sequences and the reference sequences deposited in the GenBank database. In addition to this, the query was also done in BOLD (Barcode of Life Database).

3.3.6 Data Analysis

Before analysis, the percentage incidence and severity data was transformed by arcsine transformation (Gomez and Gomez, 1984). The data were processed using IBM® Statistical Package for Social Sciences (SPSS) 26 to produce descriptive statistics on demographic traits, crop varieties, pest occurrence and management strategies (Pallant, 2011). The associations between the various variables were examined using chi-square tests (at the 5% level of significance), while the difference between means was determined using ANOVA (R Core Team, 2022). The nonparametric correlation was conducted in SPSS to determine the relationship between pest incidence and the frequency of insecticide application.

3.4 Results

3.4.1 Demographic characteristics of farmers in the study areas

Among the visited fields, 66% were managed by male farmers compared to 34% by their female counterparts. Kajiado County had the highest percentage of male farmers, while Nyandarua had the lowest (Table 3.2). Gender differences across the five counties were statistically significant ($\chi2 = 13.80$, df = 4, $p = 0.008$). About 42% of farmers were aged between 45 and 54 years, while only 3.1% were aged between 18–24 years. The age difference of farmers was not statistically significant ($\chi2 = 13.86$, df = 20, $p = 0.837$). About 50% and 32% of farmers had attained primary and secondary education across all counties.

Kiambu had the highest percentage of farmers with tertiary education, while Kajiado had the greatest number of respondents with no education. The differences in education levels of the respondents across different counties were statistically significant ($\chi2 = 22.0$, df = 20, $p = 0.038$). About 82% of the farmers interviewed grew onions and cabbage on a small scale (≤ 1 acre), except in Kajiado County, where the majority of the farmers planted onions on 2–4 acres of land (Table 3.2). The production of onions and cabbage on land sizes of ≥ 8 acres was only evident in Kajiado and Nakuru counties but on a small scale. The differences in land size dedicated to onion and cabbage farming were statistically significant across the counties surveyed ($\chi2 = 66.62$, df = 12, $p < 0.001$).

Table 3.2: Demographic characteristics of the respondents

Variables		Nyandarua	Nakuru	Kiambu (%)	Kajiado	Nyeri	Overall	χ2 value	Df	P value
Gender	Male	44.4	58.5	56.3	88.0	67.7	60.2	13.80	4	0.008
	Female	55.6	41.5	43.8	12.0	32.4	39.8			
Age of the farmer (years)	18-24	2.2	3.2	3.1	4.0	0.0	2.5	13.86	20	0.837
	25-34	4.4	6.4	3.1	12.0	5.9	6.0			
	35-44	22.2	17.5	25.0	20.0	23.5	21.1			
	45-54	35.6	38.1	31.3	44.0	52.9	39.7			
	55-64	26.7	22.2	18.8	16.0	14.7	20.6			
	≥ 65	8.9	12.7	18.8	4.0	2.9	10.1			
Education level	None	0.0	0.0	0.0	8.0	2.9	1.5	22.00	12	0.038
	Primary	44.2	50.0	35.5	64.0	50.0	48.2			
	Secondary	39.5	35.5	32.3	24.0	38.2	34.8			
	Tertiary	16.3	14.5	32.3	4.0	8.8	15.4			
Land area dedicated to crop production (acres)	≤ 1	77.8	72.3	78.1	20.0	91.2	71.1	66.62	12	<0.001
	2-4	20.0	24.6	21.9	44.0	8.8	22.9			
	5-7	2.2	0.0	0.0	24.0	0.0	3.5			
	≥ 8	0.0	3.1	0.0	12.0	0.0	2.5			

3.4.2 Vegetable varieties grown

Cabbage varieties Gloria F1, Pructor F1 and Victoria F1 were the most grown by farmers in the study area whereas Malbec F1, Red Creole, Mang'ola, Red Coach and Russet F1, were the most common in onion varieties grown. For cabbage varieties, Gloria F1 was the most grown in Nyandarua and Nakuru counties, whereas Pructor F1 was mostly grown in Kiambu (Figure 3.3). The Victoria F1 variety was preferred more by farmers in Nakuru; however, the degrees of cultivation of the three cabbage varieties were statistically similar ($f = 1.789$, $df = 8$, $p = 0.246$). The local cabbage variety, Copenhagen, was grown more in Nyandarua County than in Kiambu. The production of other local and hybrid varieties was observed in 4.3% of the farmers across the counties. In general, there were significant differences in the type of varieties of cabbage and onion grown across the three counties ($\chi2 = 48.766$, $df = 26$, $p = 0.004$).

For onion varieties, Malbec F1 was the most dominant in Nakuru County, grown by about 35% of farmers and second dominant in Nyeri County after Russet, while the Red Creole variety was only cultivated in Nakuru County on a large scale. There was also a strong preference for Jambar F1 and Africa Red F1 in Nakuru and Nyeri County, respectively. A white-bulb onion variety (Texas grano F1) was only grown in Nakuru and Nyeri Counties, whereas the local variety (Mang'ola) was the most common in Kajiado County, followed by the hybrid variety Red Coach F1. The spring onion varieties: green bunching and white Lisbon, were only grown in Nyandarua and Nakuru Counties (Figure 3.3). The difference in the preferred onion varieties across the four counties was statistically significant (χ^2 = 169.048, df = 48, $p < 0.001$).

Farmers also expressed concerns over the numerous challenges impacting vegetable farming. About 95.5% of farmers mentioned crop pests and diseases as the most critical challenges influencing onion and cabbage farming, whereas 54.2% attributed low vegetable yield to elevated costs of pesticides and fertilizers. Many farmers located away from major roads cited poor markets as a key challenge hindering vegetable farming (Figure 3.2). About a quarter of the sampled farmers were concerned over the lack of government support in terms of input subsidies and access to markets, while others expressed concern over seed quality/costs and labour (Figure 3.2).

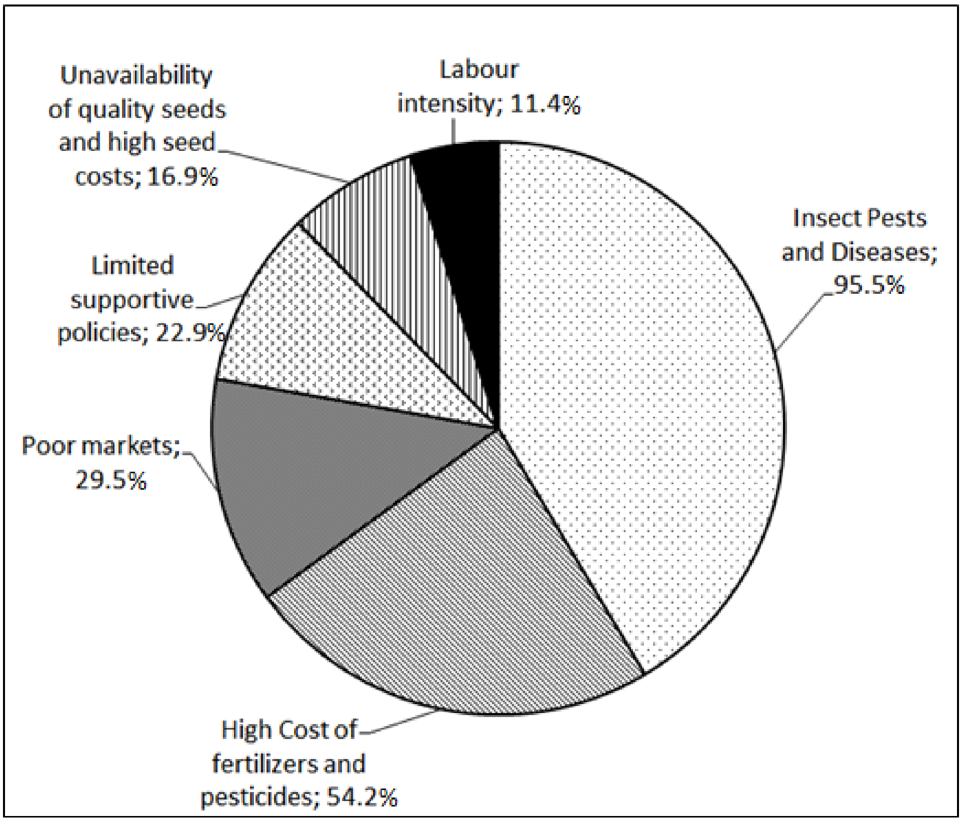

Figure 3.2: A summary of major constraints to vegetable farming in Kenya

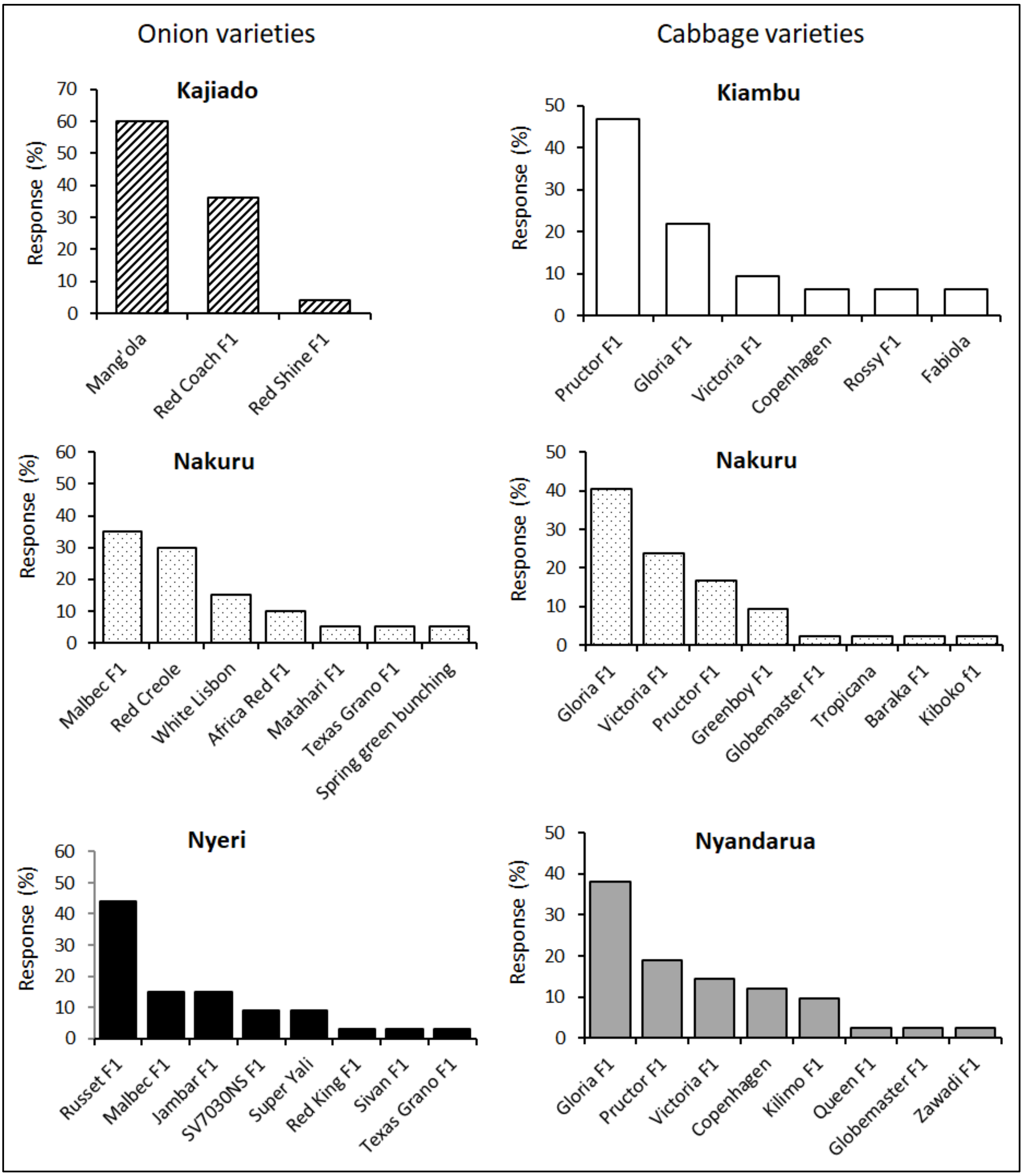

Figure 3.3: Major onion and cabbage varieties grown in Nakuru, Kajiado, Nyeri, Nyandarua and Kiambu Counties

3.4.3 Occurrence, incidence and severity of soil-dwelling insect pests

The study revealed three soil-borne pests in cabbage-growing AEZ: cabbage root flies (*Delia platura*), white grubs (*Maladera* sp.), and wireworms (*Agriotes* sp.), shown in Figures 3.4 and 3.5. Cabbage root flies had the highest occurrence of 14.0% and 12.5% in the upper highland AEZs (UH1) in Nyandarua and Kiambu Counties, respectively. White grubs and wireworms had a low occurrence of 6.4% and 5.4% in upper highland AEZs (UH1 and UH2) in Nyandarua, Nakuru, and Kiambu Counties. In onion fields, *Atherigona orientalis* was the most prevalent in lower and upper highland AEZs (UH2, LH3, and LH4) in Nakuru and Nyeri Counties, with the highest occurrences of 35.0% and 35.3%, respectively (Figure 3.5). Sap beetles were also identified on mature bulb onions of Nyeri County (UH2). The influence of AEZ on pest occurrence was statistically significant ($\chi 2$ = 41.134, df = 20, p = 0.004). Despite being a central onion-growing zone, the study did not reveal any soil-borne insect pests of economic importance in Kajiado.

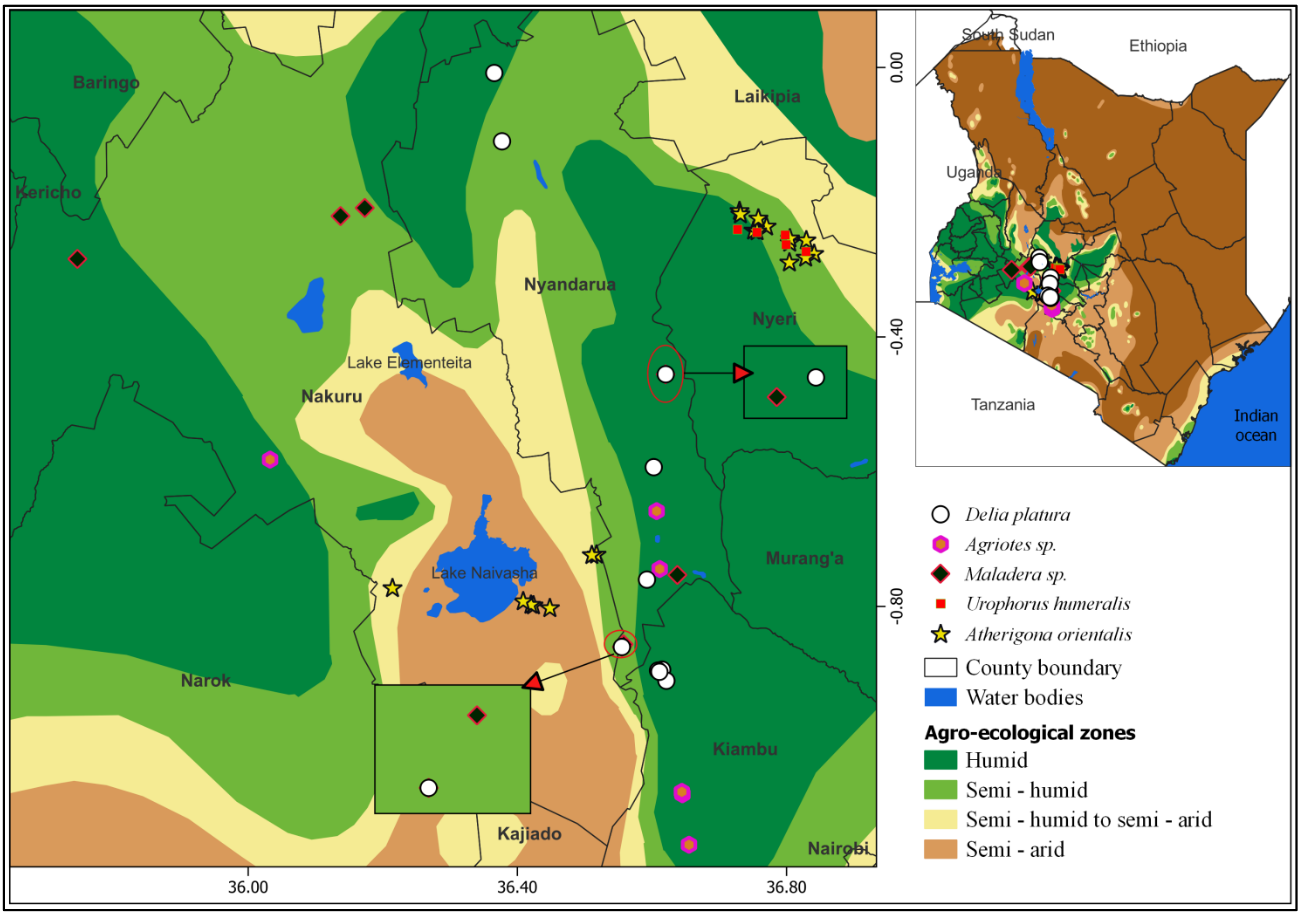

Figure 3.4: Distribution of soil-dwelling insect pests across various cabbage and onion growing agro-ecological zones of Kenya

Note: the abbreviation "sp." refers to one species identified to the genus level

Delia platura had a significantly high incidence in cabbage farms in Nyandarua County compared to Kiambu (t = 1.892, df = 8, p = 0.041). *Maladera* sp. and *Agriotes* sp. had a relatively low incidence in Nyandarua, Nakuru, and Kiambu Counties with a statistically similar occurrence across the three counties (t = 0.098, df = 11, p = 0.92). *Atherigona orientalis* had a significantly high incidence in Nakuru than in Nyeri County (t = 3.222, df = 8, p = 0.01), whereas, sap beetles were only detected in Nyeri with an incidence of 11.2% (Figure 3.5). The incidence of sap beetles and onion flies in Nyeri County was statistically similar (t = 2.131, df = 15, p = 0.296).

In cabbage fields, white grubs had the highest damage index in Nyandarua, Kiambu and Nakuru Counties compared to wireworms, but the difference was not statistically significant (t = 2.228, df = 10, p = 0.149). The cabbage root fly, only detected in Nyandarua and Kiambu Counties, had the lowest damage score ranging from 1.3 to 2.2 (Figure 3.5); however, the severity of infestation was higher in Nyandarua County (t = 2.447, df = 6, p = 0.022). In onion fields, onion flies had a significantly higher damage score of 4.8 in Nyeri County than 3.9 in Nakuru (t = 2.306, df = 8, p = 0.04). Sap beetles also had a significantly high damage score in Nyeri County, which occurred in association with the fusarium basal rot of onions. The damage index of sap beetles and onion flies in Nyeri County was statistically similar (t = 2.447, df = 6, p = 0.608). The interaction of onion flies, sap beetles and fusarium basal rot of onions resulted in severe wilting and dead plants (Figure 3.5).

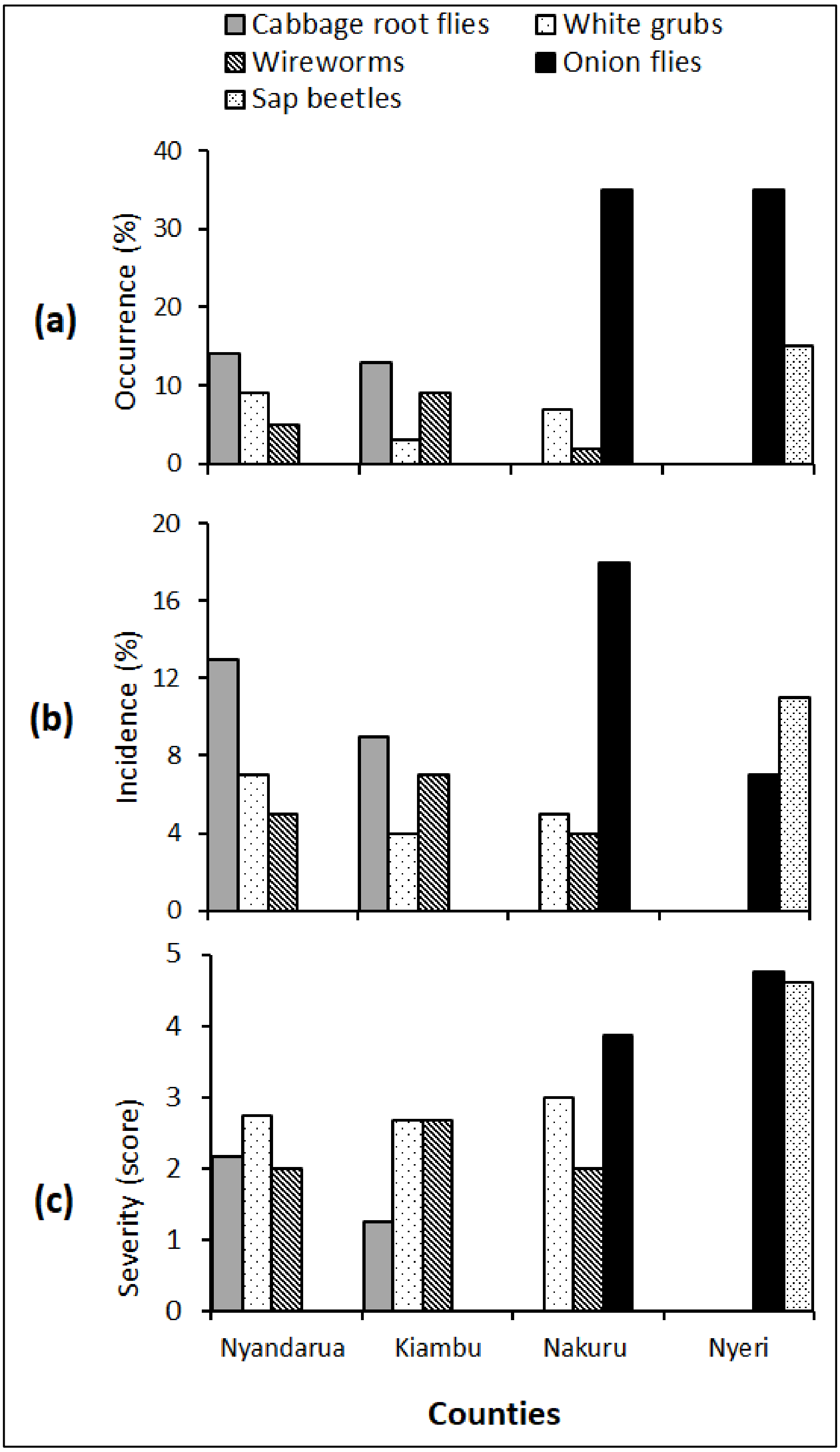

Figure 3.5: The occurrence (a), incidence (b) and severity (c) of soil-dwelling insect pests of cabbage and onion in Nyandarua, Kiambu, Nakuru and Nyeri Counties.

Note: Kajiado was excluded from Figure 3.5 because the study did not record any incidences of soil-borne insect pests of onion in the county

3.4.4 Morphological characteristics of insects associated with cabbage and onions

Wireworms (*Agriotes* spp.) – the body is straight, 29 mm long, and dark yellow in colour; nine abdominal segments are visible dorsally, with the ninth segment terminating in a blunt point with two "eye spots". Setae are centrally located on the dorsum of the 9^{th} segment while the 10^{th} segment bears the anus and lies ventrad to the 9th segment. The frontoclypeal is lyre-shaped while the labium and maxillae are fused. The mandible bears tooth-like structures on the dorsal cutting edge (Figure 3.6 j).

White grubs (*Maladera* spp.) – the body measures 26 mm in length and 2.6 mm with a smooth yellow cranium; well-developed mesothorax legs; dark brown apex of mandible and precoileae sutures. The clypeus is subtrapezoidal, with the anterior margin slightly shorter than the posterior margin while the labrum has a medial ridge. The first to third abdominal segments are slightly thicker than the other abdominal segments while the teges of raster make up about half of the posterior half (Figure 3.6 k).

Sap beetles (*Urophorus humeralis*) – the head is broad but narrower than the pronotum while mandibles have tooth-like structures on the inner side behind the apex. The elytra are truncate with three exposed chitinised dorsal segments and apically located yellow patches, while the prothorax is transverse almost as wide as the elytra. The antennae are club-shaped and moderately short (Figure 3.6 g-i).

Onion flies (*Atherigona orientalis*) – the body length is about 4 mm with sublateral black spots on the third, fourth, and fifth abdominal tergites. The antennae and palpi are dark-brown to black while the thorax is pruinose and pale grey. The ventral side bares coarse reticulation while the legs have short bristles (Figure 3.6 d-f).

Cabbage root fly (*Delia platura*) – is commonly known as seed corn flies. The adult fly is greyish, about 5 mm long and moderately setose without wing colouration. The apical posteroventral seta on the hind tibia matches the adjacent seta while the propleuron is bare. The upper calypter is larger than the lower calypter while the cercus is elongated and oval (Figure 3.6 a-c).

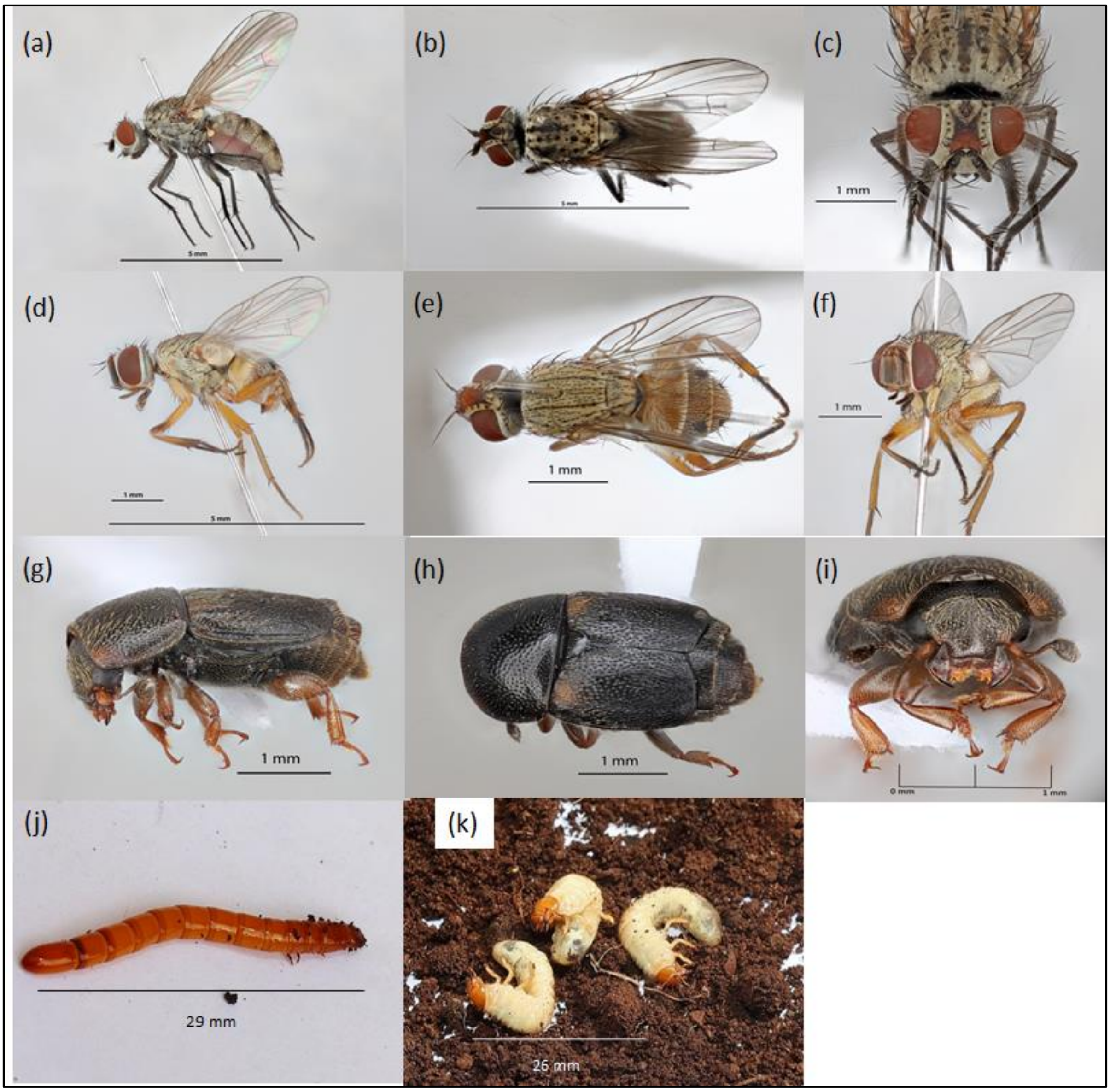

Figure 3.6: Soil-borne insect pests. *D. platura*: ventral (a), dorsal (b), and anterior views (c); *A. orientalis*: ventral (d), dorsal (e), and anterior views (f); *U. humeralis*: ventral (g), dorsal (h), and anterior views (i); *Agriotes* sp. (j), and *Maladera* sp. (k).

3.4.5 Molecular characteristics of cabbage and onion flies

The onion fly samples were positively characterized using mitochondrial COI gene, and their sequences linked to publicly available *Atherigona orientalis* COI sequences with identity similarities of ≥99 % (Table 3.3), while the cabbage fly samples linked to *Delia platura* with identity similarities of ≥97 %. For the mitochondrial COI gene region, both GenBank (NCBI) and BOLD queries gave similar identities. The D2 region of 28S large subunit rDNA corroborated the characterization achieved by the mitochondrial COI gene region, whereby the *Delia platura* linked with ≥99 % similarity (Table 3.3). However, the D2 region of 28S rDNA could only resolve the onion pests up to genus level with ≥97.56 % similarity. Figures 3.7 and 3.8 shows visual display of the bands using KETA GL imaging system.

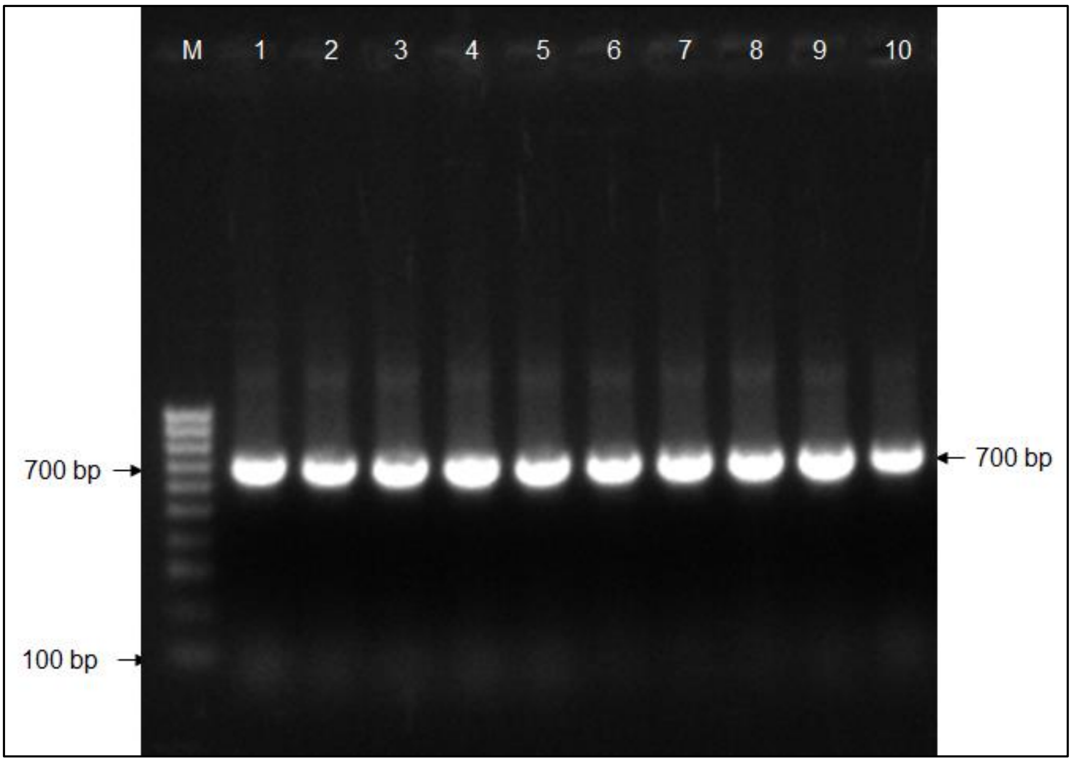

Figure 3.7: Gel electrophoresis (1.2%) of the COI gene region. **Lane M**: Hyper Ladder I (100bp, Bioline); **Lanes 1–5**: Onion fly samples (*Atherigona orientalis*); **Lanes 6–10**: Cabbage fly samples (*Delia platura*).

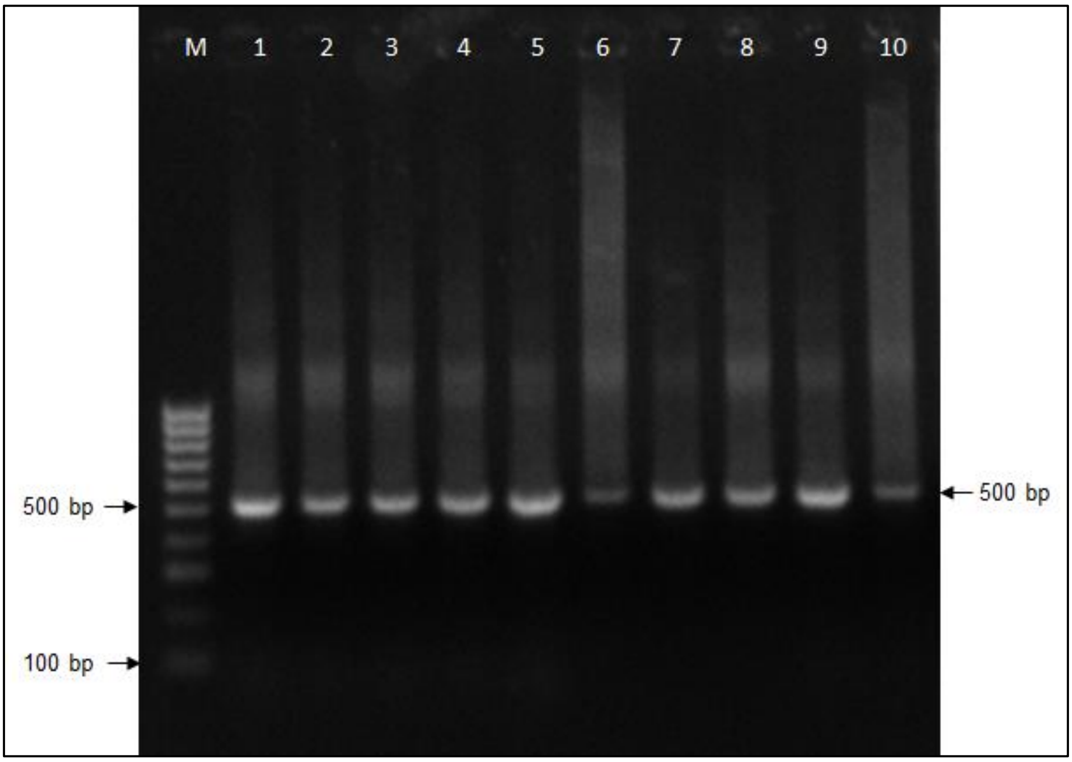

Figure 3.8: Gel electrophoresis (1.2%) of the large D2 gene region of 28S rDNA. **Lane M**: Hyper Ladder I (100bp, Bioline); **Lanes 1–5**: Onion fly samples (*Atherigona orientalis*); **Lanes 6–10**: Cabbage fly samples (*Delia platura*).

Table 3.3: Characterization of onion and cabbage pests using the mitochondrial COI and 28S rDNA domain 2 (D2) gene regions

Sample	ID from GenBank	Marker	Gene region	Accession no. (COI)	ID % (COI)	Marker	Gene region	Accession no. (28S rDNA)	ID % (28S rDNA)	Source	BOLD ID	BOLD ID%
DF1	*Atherigona orientalis*	LepF1/R1	COI	MG708349.1	99.66	LepD2	28S rDNA	KJ476333.1	97.56	Onions	*Atherigona orientalis*	100
DF2	*Atherigona orientalis*	LepF1/R1	COI	MG708350.1	99.37	LepD2	28S rDNA	KJ476333.1	97.2	Onions	*Atherigona orientalis*	100
DF3	*Atherigona orientalis*	LepF1/R1	COI	MG708349.1	100	LepD2	28S rDNA	KJ476333.1	97.57	Onions	*Atherigona orientalis*	100
DF4	*Atherigona orientalis*	LepF1/R1	COI	MG708350.1	100	LepD2	28S rDNA	KJ476333.1	97.02	Onions	*Atherigona orientalis*	100
DF5	*Atherigona orientalis*	LepF1/R1	COI	MG708349.1	99.85	LepD2	28S rDNA	KJ476333.1	97.55	Onions	*Atherigona orientalis*	100
CF1	*Delia platura*	LepF1/R1	COI	KU496718.1	97.87	LepD2	28S rDNA	FJ025507.1	99.53	Cabbage	*Delia platura*	97.94
CF2	*Delia platura*	LepF1/R1	COI	KU496718.1	97.56	LepD2	28S rDNA	FJ025507.1	99.77	Cabbage	*Delia platura*	97.94
CF3	*Delia platura*	LepF1/R1	COI	KU496718.1	97.87	LepD2	28S rDNA	FJ025507.1	99.09	Cabbage	*Delia platura*	97.94
CF4	*Delia platura*	LepF1/R1	COI	MG673923.1	97.38	LepD2	28S rDNA	FJ025507.1	100	Cabbage	*Delia platura*	97.94
CF5	*Delia platura*	LepF1/R1	COI	KU496718.1	97.72	LepD2	28S rDNA	FJ025507.1	99.54	Cabbage	*Delia platura*	97.94

3.4.6 Factors influencing pest incidence and damage severity

The education level of farmers was weakly positively correlated with the incidence and severity of soil-borne pests in onions and cabbage (Table 3.4). Agro-ecological zones were moderately correlated with pest incidence, while altitude had medium and significant negative relationships with pest incidence and severity. Nevertheless, the mono-cropping system had a weak positive but significant relationship with the damage index compared to a mixed-cropping system that exhibited a non-significant relationship. The level of farmers' awareness of soil-borne pests had a significant negative association with pest incidence and damage index. Pesticide application frequency had weak correlations with pest incidence and severity. Moreover, the pest incidence and damage index had a weak but significant negative correlation with crop yield.

3.4.7 Management of insect pests

About 95% of cabbage and onion farmers across the five counties relied on chemical pesticides to control insect pests (Figure 3.9). In addition to chemical pesticides, about half of the farmers demonstrated knowledge and utilization of crop rotation in the management of both insect pests and diseases of cabbage and onions. Other methods of insect pest management included rogueing, elimination of damaged crop residues and destruction of weed hosts (Figure 3.9). Generally, the application of botanical pesticides and traditional pest management approaches was limited.

Table 3.4: Correlation between socio-economic factors and the incidence and damage severity of soil-borne pests of soil-borne pests

Explanatory variables	Correlation Parameter Estimates	
	Incidence	Damage Severity
Education level	0.168	0.025
Agro-ecological zone (AEZ)	0.463**	0.203
Altitude	-0.350*	-0.557**
Growth stage	0.046	-0.119
Cropping system	0.241	0.324*
Awareness of soil-borne pests	-0.317*	-0.487**
Frequency of pesticide application	-0.100	0.022
Yield	-0.323*	-0.441**

Notes: ***, ** and * denotes the statistical significance of variables at 0.001, 0.01 and 0.05 p levels, respectively

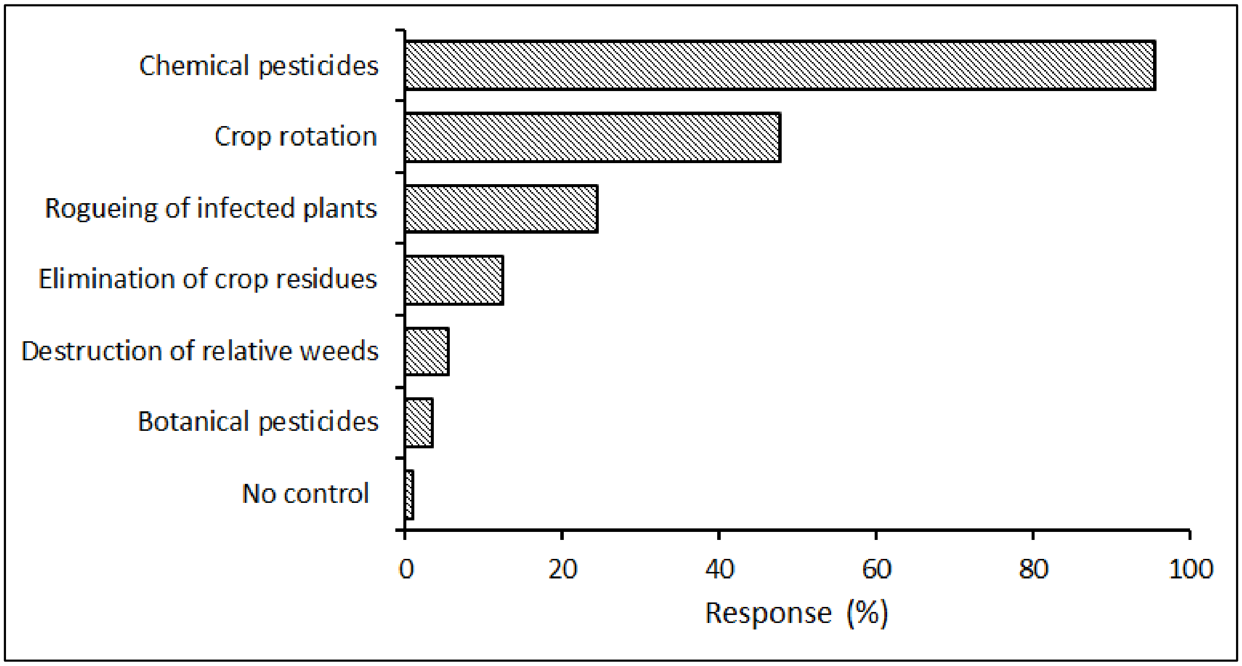

Figure 3.9: Pest management approaches used by cabbage and onion farmers in the study area

Alpha-cypermethrin, lambda-cyhalothrin, imidacloprid, beta-cyfluthrin, and acetamiprid were the most common insecticides applied by farmers in Nyandarua, Nakuru, and Kiambu Counties (Figure 3.10). Although insecticides were mostly used by farmers with primary and secondary school education, there was no significant relationship between the choice of chemicals and level of education ($\chi 2$ = 41.573, df = 22, p = 0.07). Chemicals with profenofos and cypermethrin were also common in Kiambu and Nakuru, while beta-cyfluthrin l + chlorpyriphos were only used in Kiambu (Figure 3.10). Men were more likely to use insecticides than women but the relationship was not statistically significant ($\chi 2$ = 9.920, df = 11, p = 0.537). Nonetheless, about 28% and 10% of farmers in Nakuru and Nyandarua Counties were unaware of the insecticides used in pest management. The difference in chemical use across the three cabbage-growing counties was statistically significant ($\chi 2$ = 54.868, df = 22, $p < 0.001$).

In onion fields, insecticides with profenofos and cypermethrin as active components were the most used across the three counties: Nakuru, Kajiado, and Nyeri, whereas Alpha-Cypermethrin was predominantly used in Nyeri and Nakuru (Figure 3.10). Lambda-cyhalothrin was also common across the three counties but in a low proportion, while Carbosulfan, chlorantraniliprole, abamectin, and acetamiprid were common in Kajiado (Figure 3.10). About

11%, 4% and 3% of farmers in Nakuru, Kajiado and Nyeri Counties, respectively, did not apply pesticides, while approximately 21.1% of farmers in Nakuru were unaware of the names of chemicals used (Figure 3.10). There was no statistically significant influence of gender and education on the choice of insecticides in onion fields: $\chi2 = 13.499$, df = 13, p = 0.410 and $\chi2 = 39.426$, df = 39, p = 0.451, respectively. Generally, the variation in the chemicals used across the onion-growing counties was statistically significant ($\chi2 = 71.462$, df = 26, $p < 0.001$).

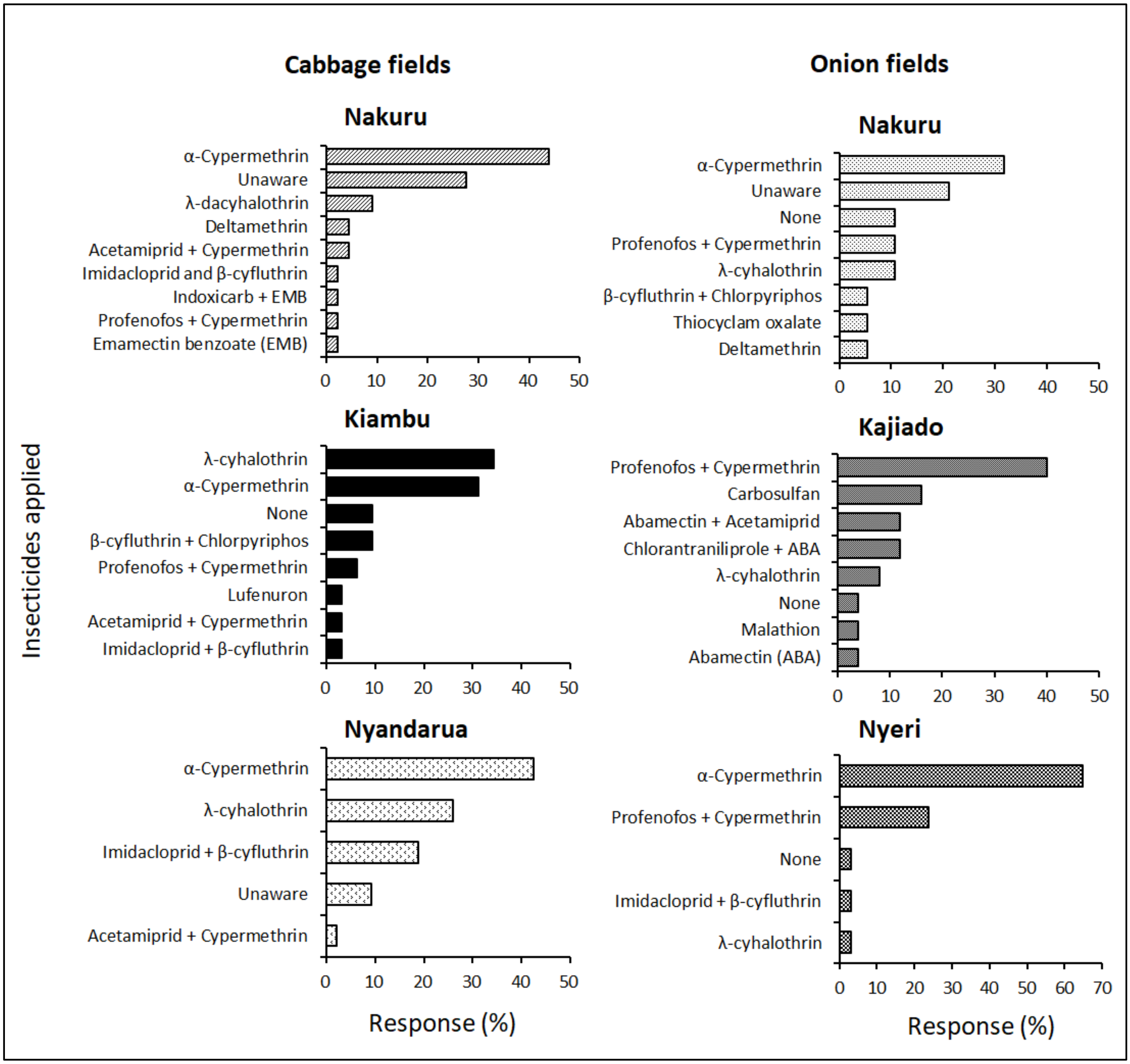

Figure 3.10: Insecticides used to control insect pests in cabbage and onions farming systems in Nakuru, Kiambu, Nyandarua, Kajiado and Nyeri Counties

3.4.8 Frequency of pesticide use

Majority of farmers applied chemical pesticides either weekly or every two weeks. About 96% of farmers in Kajiado applied pesticides weekly, while the bi-monthly application was most common in the other four counties: Nyandarua, Nakuru, Kiambu and Nyeri (Figure 3.11). Although farmers with primary school education were more likely to apply insecticides weekly, the level of education did not have a statistically significant influence on the frequency of chemical application in cabbage ($\chi2 = 18.472$, df = 12, p = 0.102) and onion fields ($\chi2 = 9.139$, df = 12, p = 0.691). In cabbage farming counties AEZ did not influence the frequency of insecticide application ($\chi2 = 38.644$, df = 30, p = 0.134) whereas in onion fields, AEZ had a significant influence on chemical use ($\chi2 = 99.949$, df = 20, $p < 0.001$). Nonetheless, there was no clear correlation between frequent chemical application and reduced incidence of soil-borne pests (r = – 0.053, p = 0.729).

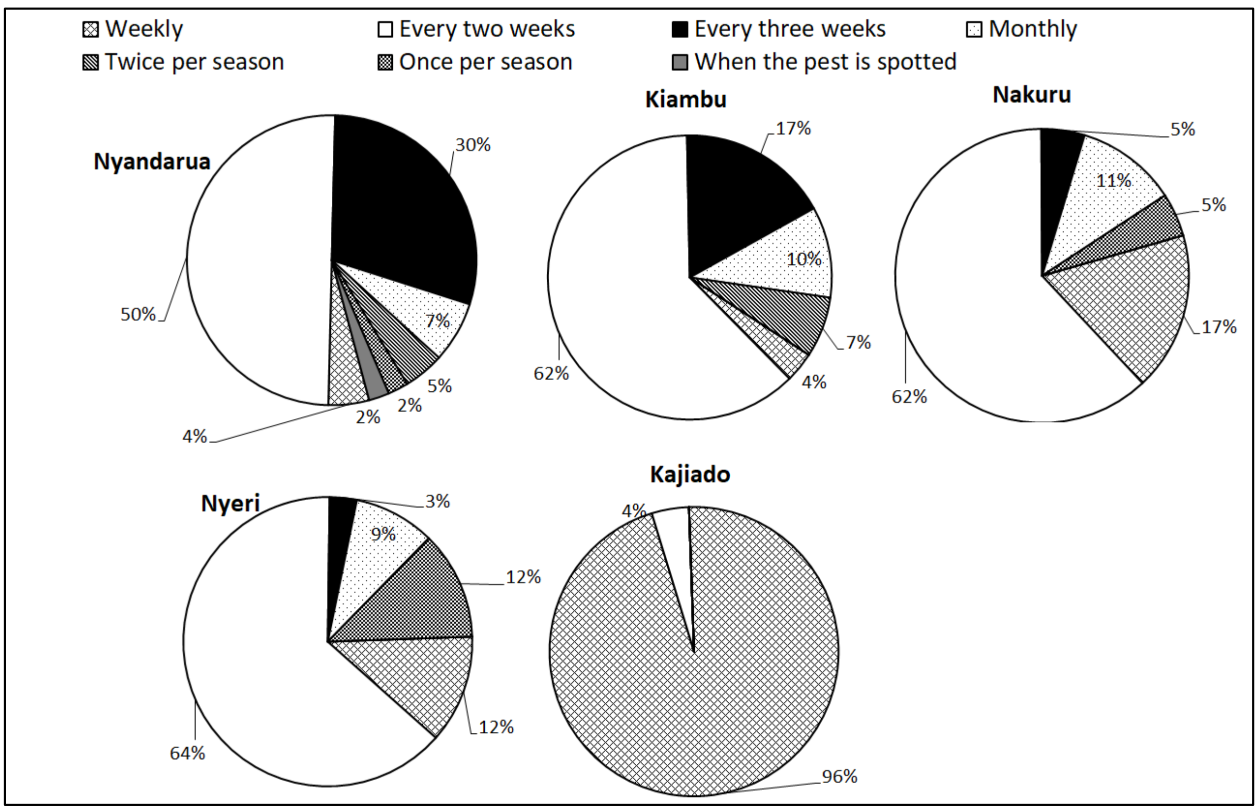

Figure 3.11: Frequency of pesticide application by cabbage and onion farmers in Nyandarua, Kiambu, Nakuru, Nyeri and Kajiado Counties

3.5 Discussion

3.5.1 Socio-economic characteristics of vegetable farmers

The study has demonstrated that male farmers are more involved in vegetable farming compared to their female counterparts. That could be attributed to socio-economic barriers that limit equitable access to resources such as land, finance and market. This gender disparity in agriculture has also been previously reported by Slavchevska, (2015) and attributed to cultural, social, and economic barriers to female farmers. The high involvement of male farmers compared to their female counterparts could also be attributed to higher returns in onion and cabbage production compared to other production systems (Anderson *et al.*, 2021). The study found that a large percentage of vegetable farmers are aged 45 – 54 years, with only 8.5% participation of young people aged below 34 in farming. The low participation of young people can be attributed to limited access to capital and land, negative attitude towards agriculture and limited agricultural knowledge and skills (Afande *et al.*, 2015). These results are consistent with past studies that reported lower levels of youth engagement in agriculture (Noorani, 2015; Wairegi *et al.*, 2018), and the World Bank report (Filmer & Fox, 2014) which revealed that only 10% of the 13.7 million youths are directly involved in agriculture. It should also be noted that most youths within the age bracket of 18 – 34 are still engaged in education, thus limited time to participate in agriculture. Nevertheless, there is a need to transform the African agriculture sector to make it more attractive to the younger generation through increasing access to capital and land, mechanization and digitization using science and technology (Anyidoho *et al.*, 2012).

The study revealed that about half of vegetable farmers have a primary school education. This corresponds with previous studies in Ghana which reported low education as the major hindrance to the adoption of cocoa farming technologies (Kyei & Foli, 2011). Farmers' level of education has a substantial influence on the choice of crop variety, time of planting, crop husbandry practices, pest management practices and technology adoption, which influence the general crop productivity (Mariano *et al.*, 2012; Silvestri *et al.*, 2012; García de Jalón *et al.*, 2015; Mwangi & Kariuki, 2015; Kinuthia *et al.*, 2018; Mutanyagwa *et al.*, 2018). For instance, in this study, farmers with primary school education levels tended to apply pesticides weekly using excessive doses, compared to those who had attained tertiary education.

There was a high preference for hybrid varieties by farmers in the study area. This has been previously reported in Kenya and could be attributed to their high-yielding ability, compactness, early maturity, disease resistance, long shelf life and strong pungency (Wambani *et al.*, 2004; Arim *et al.*, 2019). Nonetheless, some farmers expressed a preference for the Copenhagen variety, a low-yielding local variety that is affordable, especially for domestic use. For example, the production of the local onion variety, Mangola, by farmers in Kajiado County could be largely attributed to ease of accessibility at a low cost from the neighbouring country, Tanzania. The higher percentage of farmers engaged in red onion production compared to white onion could be largely attributed to higher market demand and the presence of favourable production conditions in the study areas (Birithia *et al.*, 2021).

3.5.2 Soil-borne vegetable pests and their management

The present study demonstrated that onion fly (*A. orientalis*) and sap beetles (*U. humeralis*) are widespread soil-borne pests of onion in Nakuru and Nyeri Counties, whereas cabbage root fly/seedcorn flies (*Delia platura*), white grubs (*Maladera* sp.), and wireworms (*Agriotes* sp.) are the most serious pests cabbage in Nakuru, Nyandarua and Kiambu Counties. This is the first report of *A. orientalis* and *U. humeralis* as emerging soil-borne pests of onions. Although *Delia platura*, *Maladera* sp., and *Agriotes* sp. have been previously reported as common pests of vegetables in temperate and tropical regions (Blackshaw & Kerry, 2002; Staudacher *et al.*, 2013; Johnson *et al.*, 2016; Nyamwasa *et al.*, 2017, 2018), the current study reveals moderate to high damage potentials in Kenya's cabbage growing regions.

Delia platura had a moderate occurrence but with slight damage and infestation in major cases. The low infestation could be attributed to the frequent use of chemical pesticides by farmers in the study area. Previous observations have reported moderate to high damage by *D. platura*, *D. radicum* and *D. antiqua* in cabbage and onion fields, especially in Nearctic and Palaearctic regions (Wilson *et al.*, 2015). The presence of adult *D. platura* in cabbage fields with no signs of larval infestation could signify the third-generation flies which emerge when the crop is in physiological maturity, thus causing the least or uneconomical damage (Soroka *et al.*, 2001; Capinera, 2008; Collier & Finch, 2020). Furthermore, the application of broad-spectrum pesticides and unfavourable climatic conditions, such as dynamic temperatures might have reduced egg production, enhanced egg predation and caused insect starvation (Capinera, 2008).

The current study found a high incidence of onion fly with damage index scores of 4 (considerable damage and infestation) and 5 (very high infestation levels/wilted and dead plants) in lower and upper highland AEZs (UH2, LH3 and LH4). This aligns with previous cases reported in Pakistan, where infestation ranges from 25% to 85% in melon fruits (Chughtai & Khan, 1985). *Atherigona orientalis* is a polyphagous pest that feeds on plant materials damaged by other pests, but it can also be a primary pest in Solanaceae vegetables. For instance, *A. orientalis* is a major pest of *Capsicum annuum* L. in Nigeria, causing severe damage to both ripe and unripe pepper fruits, whereas the pest has been found on tomatoes and garlic chives in Korea (Ogbalu *et al.*, 2005; Suh & Kwon, 2016). Although *A. orientali*s was first reported in Kenya in Kilifi County in 1999 by Robert S. Copeland, this is the first record of the insect as a major pest of onion in the country. *Atherigona orientali*s has a wide distribution globally and has previously been reported in Afrotropical, Nearctic, Neotropical, Indo-Malayan, and Australasian regions as a major pest of cauliflower and cabbage *Brassica oleracea* L., orange *Citrus sinensis* L., bell pepper *Capsicum annuum* L., tomato *Lycopersicon esculentum* L., melon *Cucumis melo* L., *Sorghum bicolor* L. and *Phaseolus* spp. (Suh & Kwon, 2016; CABI, 2022a; GBIF, 2022). The absence of onion root flies in the lower midland zone (V) of Kenya can be attributed to the semi-arid agro-ecological conditions characterized by low altitude (970–1390 m), low annual mean rainfall (420–520 mm), and high daily temperature range of 23–28 ^{o}C, which may result in insect mortality due to desiccation (Jaetzold & Schmidt, 1983).

The findings demonstrated that while white grubs are limited in distribution, they can cause severe stunting and wilting under heavy infestation. Previous studies have reported severe leaf damage by the adults and larvae to a broad range of horticultural crops and forestry (Held & Ray, 2009; Eckman, 2015). The current study did not detect any damage by the adult beetles, possibly due to their nocturnal feeding nature (Eckman, 2015). It should be noted that white grubs have been declared a biosecurity threat with a history of biological invasion (Ahrens, 2007). The invasive species have also been reported in the United States, the Republic of Georgia, Turkey, Canada, and Rwanda (Ahrens, 2007; Cutler & Rogers, 2009; Nyamwasa *et al.*, 2017).

The study also demonstrated that wireworms are emerging pests of cabbage with moderate to high crop damage especially in Kiambu. Previous studies have reported over 39 species from 21 genera of wireworms attacking potatoes, carrots, and sugar beets, especially in Europe and

America (Ester & Huiting, 2007; Vernon *et al.*, 2016; Kroschel *et al.*, 2020; Poggi *et al.*, 2021; Vernon & van Herk, 2022). Although the damage intensity of wireworms on cruciferous crops has not been quantified in SSA, global studies have reported severe feeding damage on sweet potatoes (Andrews *et al.*, 2008; Willis *et al.*, 2010; Abney & Kennedy, 2011; Vernon *et al.*, 2016; Vernon & van Herk, 2022). The consequent root damage hampers the absorption of nutrients, leading to stunting, reddish or purple leaf colouration and the narrowing of leaves (Karthika *et al.*, 2020). Wireworms can cause 100% crop damage when an infestation occurs during the seedling stage, leading to poor crop stands (Ester & Huiting, 2007).

Sap beetles, *Urophorus humeralis* (Coleoptera: Nitidulidae) were detected in Kieni (upper Highland), causing severe rot and wilting (up to 100% crop damage) on mature onion bulbs. Similar results have been reported on the strawberry sap beetle, where crop damage on semi-ripe and ripe fruits was 100%, while unripe fruits showed 82.3% damage after 48 hours of exposure (Fornari *et al.*, 2013). Morphologically, the pineapple sap beetle is blackish with shortened elytra, exposing three abdominal segments of 3.5–5 mm, segmented antennae with a distal club, and visible abdominal sternites. The pest was primarily detected alongside onion fly, acting as a secondary pest of field onions in some cases. *Urophorus humeralis* was also associated with fusarium basal rot of onions (*Fusarium oxysporum* f. sp. *cepae*), indicating that the pest either attacks rotting onions due to fusarium basal rot or is involved in the transmission of the disease. This observation aligns with Konam & Guest (2004), who found that Scolytidae and Nitidulidae beetles were attracted to *Phytophthora palmivora* disease lesions and facilitated disease transmission. Moreover, sap beetles are attracted to volatile compounds produced by *Fusarium verticillioides* in maize (Bartelt & Wicklow, 1999; Konam & Guest, 2004), highlighting the need to establish the interaction between *U. humeralis* and onion crops infested with onion fly and *Fusarium* basal rot.

The current study revealed that approximately 95% of farmers in the study area rely on chemical pesticides to control pests. This is in agreement with previous studies which revealed that 70 – 95% of Kenyan farmers use synthetic pesticides for the management of vegetable and fruit pests (Kilalo *et al.*, 2009). The absence of soil-borne onion insects in Oloitokitok could be largely attributed to the high frequency of pesticide application observed during the study, whereby 96% of the farmers applied pesticides weekly and in excess doses. The high frequency of application in excessive dosage has been previously reported (Mutuku *et al.*, 2013; Mulati *et al.*, 2018) and could be due to the ease of access to chemical pesticides at a low cost from neighbouring Tanzania. It was noted that some of the commonly used pesticides such as pyrethroids (lambda-cyhalothrin, alpha-cypermethrin, and beta-cyfluthrin) and neonicotinoids (imidacloprid) are moderately hazardous to human health and the environment, according to the WHO classification (Table 3.5) (PCPB, 2018). Moreover, 21 – 27% of the farmers interviewed were unaware of the specific product names of the pesticides used in their farms. Lack of knowledge regarding pesticides greatly contributes to excessive use, which could lead to pesticide toxicity, pest resistance, and ecosystem damage.

Previous studies have reported high usage of pyrethroids and organophosphates in controlling insect pests in Kenya (Kilalo *et al.*, 2009; Macharia *et al.*, 2013; Nguetti *et al.*, 2018; Omwenga *et al.*, 2021). Poor pesticide use characterized by excessive dosage and short frequencies of application could lead to pesticide resistance. For example, farmers in Kajiado County expressed concern over above-ground pests such as *T. tabaci*. The poor pesticide use observed in Kajiado could also be attributed to low literacy levels whereby, about three-quarters of farmers in the area had a primary school education or below. Therefore, there is a need to create awareness and build the capacity of farmers to effectively use pesticides for sustainable pest management. The absence of soil-dwelling pests in Kajiado is probably due to basin irrigation, which might have resulted in drowning and/or suffocation, and the high frequency of insecticide application.

About 47.8% also used crop rotation as an additional approach to pest management. Although, non-chemical approaches to pest management, such as rogueing of infected crops, destruction of relative weeds, use of traditional and botanical pesticides, and elimination of crop residues, are essential in the integrated management of insect pests, farmers did not leverage these approaches (Chepchirchir *et al.*, 2021). Therefore, there is a need to promote integrated pest management approaches among vegetable farmers in Kenya for the effective management of insect pests as well as crop diseases.

Table 3.5: Characterization of chemical pesticides used by farmers in major vegetable growing areas of Kenya

Active component	Product Names	WHO Class	Chemical class	Mode of Action
Lambdacyhalothrin	Duduthrin 1.75 EC, Halothrin 2.5 EC, Karate 2.5 WG, Pentagon 50 EC, Vendex 50 EC	Class II	Pyrethroid	Contact, ingestion and ovicidal action
Imidacloprid and beta-cyfluthrin	Thunder 145 OD, Buffalo 100 OD	Class II	Imidacloprid is a neonicotinoid while beta-cyfluthrin is a pyrethroid	Contact and systemic residual action
Beta-cyfluthrin + Chlorpyriphos	Betaforce 263 EC	Class II	Beta-cyfluthrin is a pyrethroid while Chlorpyriphos is an organophosphate pesticide	Contact, stomach and respiratory action
Alpha-Cypermethrin	Bestox20 EC, Tata Alpha 10 EC	Class II	Pyrethroid	Disruption of Voltage-gated sodium channel (VGSC) function
Acetamiprid + Cypermethrin	Aster Extrim 20 SL, Twiga Ace 20 SL	Class II	Acetamiprid is a chloropyridinyl neonicotinoids while Cypermethrin is a pyrethroid	Systemic, translaminar action (acetamiprid), contact & stomach action (cypermethrin)
Thiocyclam hydrogen oxalate	Taurus 500SP	Class II	Nereistoxin analogue	Contact and stomach actions
Deltamethrin	Decis 2.5 EC, Decis Forte EC 100EC	Class II	Pyrethroid ester insecticide	Ingestion and direct contact
Emamectin benzoate	ESCORT 19 EC	Class III	Avermectin	Disruption of the nerve impulses
Profenofos + Cypermethrin	Profile 440 EC, Profecron 44 EC,	Class II	Profenofos is an organophosphate insecticide while Cypermethrin is pyrethroid	Acetyl cholinesterase (AChE) inhibitor with contact and stomach action
Indoxicarb + Emamectin Benzoate	Benocarb 100SC	Class II	Indoxacarb is an oxadiazine pesticide while Emamectin Benzoate is an avermectin	Indoxicarb acts by blocking the neuronal sodium channels, while Emamectin Benzoate disrupts the nerve impulse
Lufenuron	Match 050 EC	Class III	Imidacloprid is aneonecotinoid Insect Growth Regulators	Inhibits chitin synthesis and interferes with moulting.
Chlorantraniliprole + Abamectin	Voliam Targo 063SC	Class II	Chlorantraniliprole is an anthranilamide insecticide while Abamectin is an avermectin	Chlorantraniliprole is a ryanodine receptor modulator while Abamectin is a GABA agonist
Carbosulfan	Marshal 250 EC	Class II	Carbamate insecticide	contact and stomach poison action
Abamectin	Acoster 5 EC	Class II	Avermectin insecticide	Stimulates the gamma-aminobutyric acid (GABA) system
Malathion	Oshothion 50 EC	Class II	Organophosphate insecticide	acetylcholinesterase inhibitor
Abamectin + Acetamiprid	Dudu Acelamectin 5% EC	Class II	Abamectin belongs to avermectin class while acetamiprid is a chloropyridinyl neonicotinoids	Abamectin is a GABA agonist while acetamiprid has systemic and translaminar action

Source: Pest Control Products Board of Kenya (PCPB, 2018)

3.6 Conclusion

This study demonstrated that onion fly (*A. orientalis*) are the most widespread soil-borne pests of onion, with a prevalence of 35% and severe infestation in main onion-growing counties. *Atherigona orientalis* and *Delia platura* are emerging root flies of cabbage and onions in the lower and upper highlands of Kenya. Other soil-borne pests identified across different agroecological zones were white grubs, wireworms and sap beetles, with incidences ranging from 6.7 to 11.2. The severity of the pest damage was between 1.3 and 4.6, with higher values obtained in fields affected by *D. platura*, *A. orientalis* and *U. humeralis*. It was noted that soil-dwelling pests which occurred in association, such as sap beetles and onion flies, aided in the transmission of plant diseases, especially Fusarium basal rot, causing further yield losses. Most farmers applied broad-spectrum insecticides, primarily pyrethroids and organophosphates, weekly or every two weeks, but with little success. Although 49% of the farmers used crop rotation to manage pests, integrated pest management approaches have yet to be embraced in vegetable cropping systems. Therefore, there is a need to classify the prevailing soil-dwelling pest in the context of tropical climate and to design sustainable management approaches to limit their spread.

CHAPTER FOUR

INSECTICIDAL POTENTIAL OF LIQUID FERTILIZER MADE FROM BLACK SOLDIER FLY FRASS AND PUPAE EXUVIAE AGAINST ONION FLY (*Atherigona orientalis*)

4.1 Abstract

Onion flies are polyphagous pests with high damage potential on bulb onions in the field and under storage. This study evaluated the insecticidal potential of liquid fertilizer formulations made from black soldier fly pupae exuviae and frass on onion fly larvae, as a sustainable and environmentally friendly alternative to chemical pesticides. Onion fly larvae were collected from Naivasha, Nakuru County and reared in fabricated cages for three generations before the experiment. The egg and larval stages were then exposed to black soldier fly liquid fertilizer formulations made from varying proportions of pupae exuviae and frass fertilizer under laboratory conditions. The study found a positive correlation between black soldier fly pupae exuviae rates and the mortality of onion flies. The highest activity of the black soldier fly frass fertilizer was observed on *A. orientalis* eggs, with 65% egg mortality. Additionally, liquid fertilizer formulation with 100% black soldier fly pupae exuviae caused a range of 20.9–22.4% mortalities of second-instar larvae under contact and residual exposures after 72 hours. However, larval mortality of 31.2% was achieved for the first instar larvae after 72 hours of residual exposure using formulations with >20% pupae exuviae. The effect of black soldier fly liquid fertilizer on *A. orientalis* can be attributed to chitin and chitosan, which favours the population of chitinolytic microorganisms that digests the polysaccharide chitin to disaccharide chitobiose, thus degrading shells and cuticles of eggs and larvae. The findings from this study indicate that black soldier fly liquid fertilizer has the potential as an element of integrated management of onion fly in cultivated onion as a soil amendment.

Keywords: Onion fly, black soldier fly, pupae exuviae, frass fertilizer, and liquid fertilizer

4.2 Introduction

Onion fly (*Atherigona orientalis*) is a polyphagous pest that feeds on plant materials damaged by other pests, but it can also be a primary pest of Solanaceous vegetables (Ogbalu *et al.*, 2005). Onion fly causes the most substantial damage to onions by directly foraging on young plants early in the season (McDonald *et al.*, 2004). Gravid female flies oviposit at the base of the onion plant, causing infestation at the root zone and subterranean feeding (Moretti *et al.*, 2021). Damage by first-generation onion fly ranges from 20–80% in the US (Nault *et al.*, 2006; Moretti & Nault, 2020), 50-65% in India (Gupta *et al.*, 2021) and 24.6-83.7% in Poland (Szwejda, 1982). Although mature bulbs are more resistant to onion fly infestation, mechanical damage during harvesting can cause infestations in the field, leading to post-harvest yield loss (Capinera, 2008). Wounds created by onion fly also act as pathways for infection by soil-borne fungi and bacteria. For instance, onion fly infestations increase the incidence of soft rot by creating injuries that serve as entry pathways for pathogens. The larvae may also be contaminated with the bacterium aiding in dispersal while the puparium may harbour the bacterium enabling survival under inclement conditions (McDonald *et al.*, 2002; Hoepting *et al.*, 2004; Capinera, 2008).

While broad-spectrum pesticides such as organophosphate and carbamates are primarily preferred in controlling onion flies (Joseph & Zarate, 2015), the excessive application is harmful to human and environmental health and can destabilize nature's biodiversity (Cresswell, 2011; Krupke *et al.*, 2017). For instance, studies have revealed that high levels of organophosphorus, organochlorine, and carbamate residues in the Lake Naivasha ecosystem, including fish samples, water, and water sediments, are due to runoff discharge from agricultural practices in the region. Chitin obtained from crustaceans' shells has been extensively studied to elicit the defence mechanism of plants against pests by triggering the repellent phytoalexins (Shimizu *et al.*, 2010; Shamshina *et al.*, 2020). However, crustaceans' shells are limited, and extracting chitin from the shells is expensive, limiting the potential of this compound in integrated pest management. Black soldier fly mass rearing has increased the availability of pupae exuviae and dead imago, which can be leveraged as an alternative source of chitin. Therefore, this study evaluated the insecticidal potential of liquid fertilizer formulations made from BSF pupae exuviae and frass on onion fly.

4.3 Materials and Methods

4.3.1 Source of onion flies

Onion fly larvae were initially collected from Naivasha, Nakuru County, and reared on an artificial diet for about three generations in the laboratory before the experiment. The adult flies were reared in fabricated cages and fed on sugar, yeast extract, and milk powder mixed at a ratio of 1:1:1. Water-soaked cotton balls were provided and changed frequently to maintain a clean water supply (Kayukawa *et al.*, 2007). The cages were kept in rearing chambers at 50-70% relative humidity, 24±2 ˚C, and 12 h light: 12 h dark photoperiod (Finch *et al.*, 2003; Kayukawa *et al.*, 2007). To obtain eggs, macerated bulb onions were placed in 0.5 L containers and covered with a wire mesh. The wire mesh was covered with a thin layer of sawdust to hold the laid eggs in position before hatching. Eggs laid were kept under the same environmental conditions under which they hatched for 2-3 days and began feeding on the macerated onion.

4.3.2 Preparation of the BSF liquid fertilizer

The black soldier fly frass fertilizer and pupae exuviae was prepared as described in sections 5.3.2 and 5.3.3. Black soldier fly liquid fertilizer was prepared by mixing the BSF frass fertilizer with different rates of pupae exuviae: 0%, 10%, 20%, 30%, 40%, 50%, and 100%. Each amendment was placed in absorbent sacks and submerged in a fermentation vessel with a 40 L liquid formulation made of 39 L water, 0.8 L effective microorganism culture (EMC) and 0.2 L molasses. The effective microorganism solution contained microorganisms: lactic acid bacteria, *Saccharomyces cerevisiae*, *Rhodopseudomonas* spp. and *Lactobacillus planetarium* (Beesigamukama *et al.*, 2018). The mixture was turned every seven days to increase the contact of microbes with the BSF frass amendments. The liquid fertilizer was harvested after six weeks.

4.3.3 Determination of contact toxicity of BSF liquid fertilizer against the second larval stage of *A. orientalis*

A sixty mm diameter Petri dish was cut at the bottom and fitted with a strainer (300 µm mesh size; Figure 4.1a) to make a holding cell for the larvae while dipping into the liquid fertilizer. Sets of twenty-second instar larvae (five days old) of *A. orientalis* were then restrained and dipped into the liquid fertilizers made from different amendment rates of BSF frass fertilizer and exuviae for 10, 60 and 300 seconds (Paramasivam, 2017; Ahmed *et al.*, 2020). The negative control was prepared by dipping the larvae into distilled water, whereas imidacloprid solution (6 mL/L) was used as a positive control. Each set of dipped larvae was placed on Petri dishes with perforated lids to prevent escape or suffocation (Figure 4.1b). Fleshy onion scale leaves were chopped into 5 cm–diameter pieces using a cork borer, slightly macerated and supplied to each set of the experiment as feed for the surviving larvae. The experimental set-up was done at standard rearing conditions of 50-70% relative humidity, 24±2 ˚C, and 12 hrs light: 12 hrs day photoperiods (Kayukawa *et al.*, 2007), and the rate of larval mortality was recorded after 24 hrs, 48 hrs, and 72 hrs. Each treatment was replicated six times and the experiment conducted using randomized complete block (RCB) design.

4.3.4 Determination of residual toxicity of BSF liquid fertilizer against the first and second larval stages of *A. orientalis*

Twenty grams of macerated onion was mixed with 10 ml of black soldier fly liquid fertilizer in Petri-dishes, followed by the introduction of 20-second instar larvae on each petri dish. A fine camel hair brush was used to carefully scoop the larvae into each Petri dish containing the substrate to avoid injury. The Petri dishes were covered with perforated lids (Figure 4.1b) to prevent suffocation or escape of the larvae (Kayukawa *et al.*, 2007). The negative control was prepared by mixing macerated onions with 10 ml of distilled water, whereas imidacloprid solution (6 mL/L) was used as a positive control. Petri dishes were kept at standard rearing conditions of 50-70% relative humidity, 24±2 ˚C, and 12 hrs light: 12 hrs day photoperiods (Kayukawa *et al.*, 2007). The experiment was conducted using RCB design with six replications and the rate of larval mortality assessed after 24 hrs, 48 hrs, and 72 hrs. The procedure was repeated for the first instar larvae, where mortality was only assessed after 72 hours and at pupation, and the period until pupation was recorded.

4.3.5 Assessment of ovicidal activity of BSF liquid fertilizer against *A. orientalis*

Twenty-four-hour-old eggs were collected and transferred to different filter papers of 15 mm diameter (Pineda *et al.*, 2004). The filter papers, each containing 20 eggs, were then sprayed with 5 ml BSF liquid fertilizer solutions using a hand sprayer (Pineda *et al.*, 2004; de Silva *et al.*, 2008; Rimoldi *et al.*, 2008; Baskar *et al.*, 2012). After spraying, wet filter papers containing eggs were dried in Petri dishes loaded with Whatman filter papers (Gökçe *et al.*, 2011) at room temperature and then transferred to ventilated plastic Petri dishes (Figure 4.1b). The negative control involved spraying the eggs with distilled water, whereas imidacloprid solution (6 mL/L) was used in the positive control. Fleshy onion scale leaves were chopped into 5 cm –diameter pieces using a cork borer, slightly macerated and supplied to each set of the experiment as feed for the emerging neonates. The experiment was conducted using RCB design with six replications and the number of larvae that hatched from each treatment determined after 72 hours. Egg shells were also counted under a light microscope to confirm the eclosion rate.

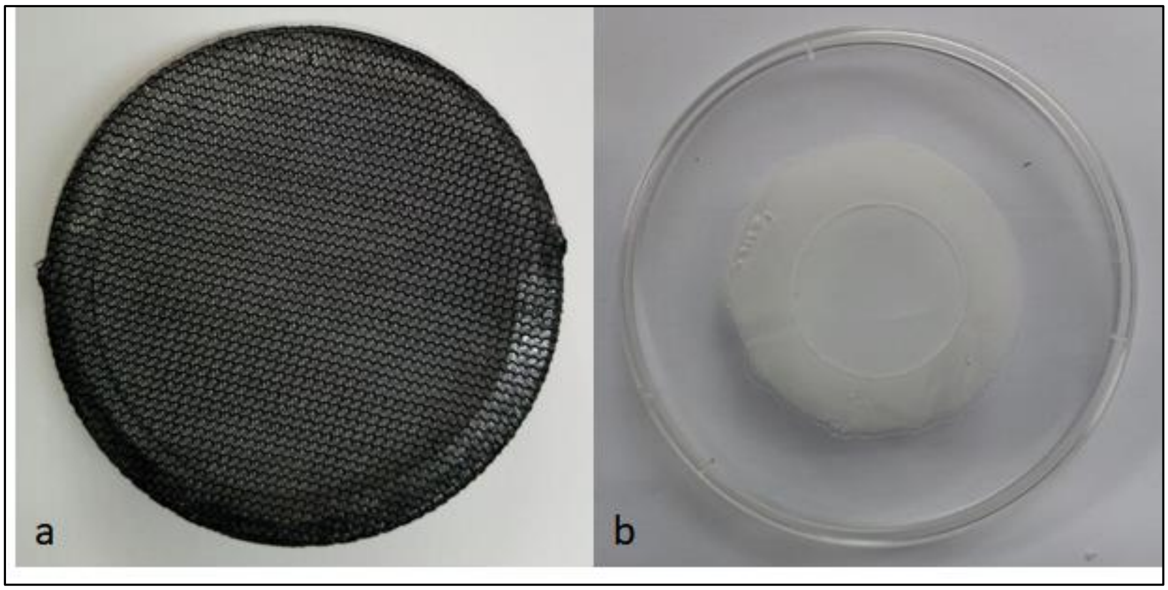

Figure 4.1: (a) Plastic Petri dish (60 mm in diameter) cut at the bottom and fitted with a strainer to make a holding cell during the dipping method; (b) Perforated Petri dish lids used for insecticidal bioassays

4.3.6 Data analysis

All recorded mortality was corrected using Abbot's equation below (Abbott, 1925):

$$\frac{x-y}{x} \times 100 = \text{Percent Mortality}$$

Where:
X = the per cent living in the check
Let Y = the per cent living in the treated plat
Then X-V = the per cent killed by the treatment

The data was subjected to a two-way ANOVA to establish the interaction between BSF liquid fertilizer formulation and mortality (R Core Team, 2022). Data normality was assessed using the Shapiro-Wilk test. The mean difference between treatments was evaluated for the significance test using Fisher's LSD at the p = 0.05 level. Survival was also analysed using generalized linear models (GLM) with binomial distributions (R Core Team, 2022).

4.4 Results

4.4.1 Effect of BSF liquid fertilizer on the second instar larvae of onion fly (*A. orientalis*) after contact exposure for 10, 60 and 300 seconds

4.4.1.1 Mortality of second instar larvae of onion fly after contact exposure to BSF liquid fertilizer for 10 seconds

Although there was a strong correlation between larval mortality and black soldier fly pupae exuviae rates in the liquid fertilizer (r = 0.842, p = 0.017), the mortality did not vary across all treatments with Exuviae. The lowest mortality was recorded after 24 hours and was similar across all liquid fertilizer formulations. Larval mortality increased slightly after 48 hours, reaching a maximum of 8.8 % for liquid fertilizer made from 100% BSF exuviae, but this was not significantly different from formulations with at least 10% exuviae (Table 4.1). Mortality of *A. orientalis* increased significantly from 24 hours to 72 hours in all treatments except for liquid fertilizer formulations without exuviae, which was constant (p = 0.90). Exposure of *A. atherigona* larvae to imidacloprid resulted in a mortality of 41.4% which was significantly higher than all other treatments. Negligible mortality was recorded in the negative control, which was corrected using Abbot's equation.

Table 4.1: Mortality of second instar larvae of onion fly after 24, 48 and 72 hours of exposure by contact/larvae dip method for 10 seconds

Formulation of BSF liquid fertilizer	Exposure time		
	24hrs	48hrs	72hrs
BSFFF+0% Exuviae	0.8b	1.06c	1.72c
BSFFF+10% Exuviae	1.7b	4.48bc	6.0bc
BSFFF+20% Exuviae	1.7b	6.19bc	8.60bc
BSFFF+30% Exuviae	1.7b	6.19bc	10.3b
BSFFF+40% Exuviae	2.5b	7.04b	9.5b
BSFFF+50% Exuviae	2.5b	6.19bc	10.3b
100% Exuviae	1.7b	8.75b	12.9b
Imidacloprid	12.6a	26.70a	41.4a
P	<0.05	<0.05	<0.05
LSD	3.64	5.15	7.54
CV%	99.06	53.05	51.28

Means followed by the same letter(s) in each column are not significantly different at p≤0.05; CV=Coefficient of Variation; LSD=Least Significant Difference at (p≤0.05); BSFFF – Black soldier fly frass fertilizer.

Note: The negative control was corrected using Abbot's equation (Abbott, 1925)

4.4.1.2 Mortality of second instar larvae of onion fly after contact exposure to BSF liquid fertilizer for 60 seconds

The BSF liquid fertilizer had a significant effect on the mortality of *A. orientalis* larvae after 72 hours ($p < 0.05$). Exposure of *A. orientalis* to liquid fertilizer formulations with ≥30% pupae exuviae resulted in a range of 16-21% larval mortality after 72 hours, but this was significantly lower than the positive control (Table 4.2). After 48 hours, liquid fertilizer formulations with ≥20% pupae exuviae had statistically similar mortality rates that ranged from 8.55% to 12.82%. The rate of BSF exuviae in the liquid fertilizer was strongly correlated with larval mortality ($r = 0.922$, $p = 0.003$); the rate of BSF exuviae in liquid fertilizer formulations significantly affected the mortality rate of *A. orientalis* (GLM: $\chi2 < 0.001$, df = 40, $p < 0.05$). The positive control imidacloprid recorded the highest larval mortalities after 24, 48, and 72hrs ($p < 0.05$), whereas negligible mortality was recorded in the negative control and was corrected using Abbot's equation (Table 4.2).

Table 4.2: Mortality of second instar larvae of onion fly after 24, 48 and 72 hours of exposure by contact/larvae dip method for 60

Formulation of BSF liquid fertilizer	Exposure time		
	24hrs	48hrs	72hrs
BSFFF+0% Exuviae	1.68b	4.27c	7.83e
BSFFF+10% Exuviae	1.68b	4.27c	10.43de
BSFFF+20% Exuviae	3.36b	8.55bc	13.91cd
BSFFF+30% Exuviae	3.36b	8.55bc	15.65bc
BSFFF+40% Exuviae	3.36b	9.40b	15.65bc
BSFFF+50% Exuviae	4.20b	11.97b	18.26bc
100% Exuviae	3.36b	12.82b	20.87b
Imidacloprid	28.57a	58.97a	77.39a
P	<0.05	<0.05	<0.05
LSD	4.84	4.68	5.17
CV%	67.05	27.02	19.69

Means followed by the same letter(s) in each column are not significantly different at p≤0.05; CV=Coefficient of Variation; LSD=Least Significant Difference at (p≤0.05); BSFFF – Black soldier fly frass fertilizer

Note: The negative control was corrected using Abbot's equation (Abbott, 1925)

4.4.1.3 Mortality of the second instar larvae of onion fly after contact exposure to BSF liquid fertilizer for 300 seconds

The BSF liquid fertilizer significantly affected the survival of *A. orientalis* larvae after five-minute contact exposure ($p < 0.05$). Exposure of second-instar larvae of onion fly to liquid fertilizer with 100% pupae exuviae resulted in the highest mortality of 21.4% after 72 hours, but this was not significantly different across all formulations with ≥20% pupae exuviae. Generally, larval mortality in treatments with chitin-fortified BSF liquid fertilizer was lowest after 24 hours and increased two- and three folds after 48 and 72 hours, respectively, except for imidacloprid, which had significantly high mortality after 24 hours after contact exposure (Table 4.3). There was a strong positive correlation between larval mortality and the rate of BSF exuviae in the liquid fertilizer ($r = 0.821$, $p = 0.024$). Increasing the level of BSF exuviae in liquid fertilizer formulations significantly affected the mortality rate of *A. orientalis* (GLM: $\chi2 < 0.05$, df = 40, $p < 0.05$). The negative control treatment showed negligible mortalities of 0.83%, 1.67% and 2.5% after 24, 48 and 72 hours, respectively, which were corrected using Abbott's formula.

Table 4.3: Mortality of second instar larvae of onion fly after 24, 48 and 72hours of exposure by contact/larvae dip method for five minutes

Formulation of BSF liquid fertilizer	Exposure time		
	24hrs	48hrs	72hrs
BSFFF+0% Exuviae	2.52c	5.08d	8.55d
BSFFF+10% Exuviae	3.36bc	6.78cd	11.11cd
BSFFF+20% Exuviae	5.88bc	11.86bc	17.09bc
BSFFF+30% Exuviae	5.88bc	12.71bc	18.80b
BSFFF+40% Exuviae	5.88bc	11.02bcd	16.24bc
BSFFF+50% Exuviae	5.88bc	13.56b	18.80b
100% Exuviae	7.56b	15.25b	21.37b
Imidacloprid	73.11a	81.36a	88.89a
P	<0.05	<0.05	<0.05
LSD	4.94	6.15	6.70
CV%	30.75	26.76	22.87

Means followed by the same letter(s) in each column are not significantly different at $p \leq 0.05$; CV=Coefficient of Variation; LSD=Least Significant Difference at ($p \leq 0.05$); BSFFF – Black soldier fly frass fertilizer

Note: The negative control was corrected using Abbot’s equation (Abbott, 1925)

4.4.2 Effect of BSF liquid fertilizer on first and second instar larvae of onion fly (*A. orientalis*) after residual exposure to BSF liquid fertilizer

4.4.2.1 Mortality of the first instar larvae of onion fly after residual exposure to BSF liquid fertilizer

Mortalities of first-instar larvae of onion fly were significantly higher than the second instar after residual exposure to BSF liquid fertilizer ($p = 0.03$). Liquid fertilizer formulations with 20% pupae exuviae recorded the highest mortality of 31.2% after 72 hours and 35% at pupation, but this was not significantly different across all treatments with ≥20% BSF pupae exuviae (Figure 4.2). There was a moderate positive correlation between larval mortality and the rate of BSF exuviae after 72 hours ($r = 0.624$, $p = 0.120$) and at pupation ($r = 0.612$, $p = 0.145$). Varying the level of BSF exuviae in liquid fertilizer formulations significantly affected the mortality rate of first instar larvae of onion fly after 72 hours (GLM: $\chi2 < 0.025$, df = 40, $p < 0.031$) and at pupation (GLM: $\chi2 < 0.001$, df = 40, $p < 0.002$). Negligible mortality was recorded in the negative control after 72 hours and at pupation, whereas imidacloprid showed 100% mortality.

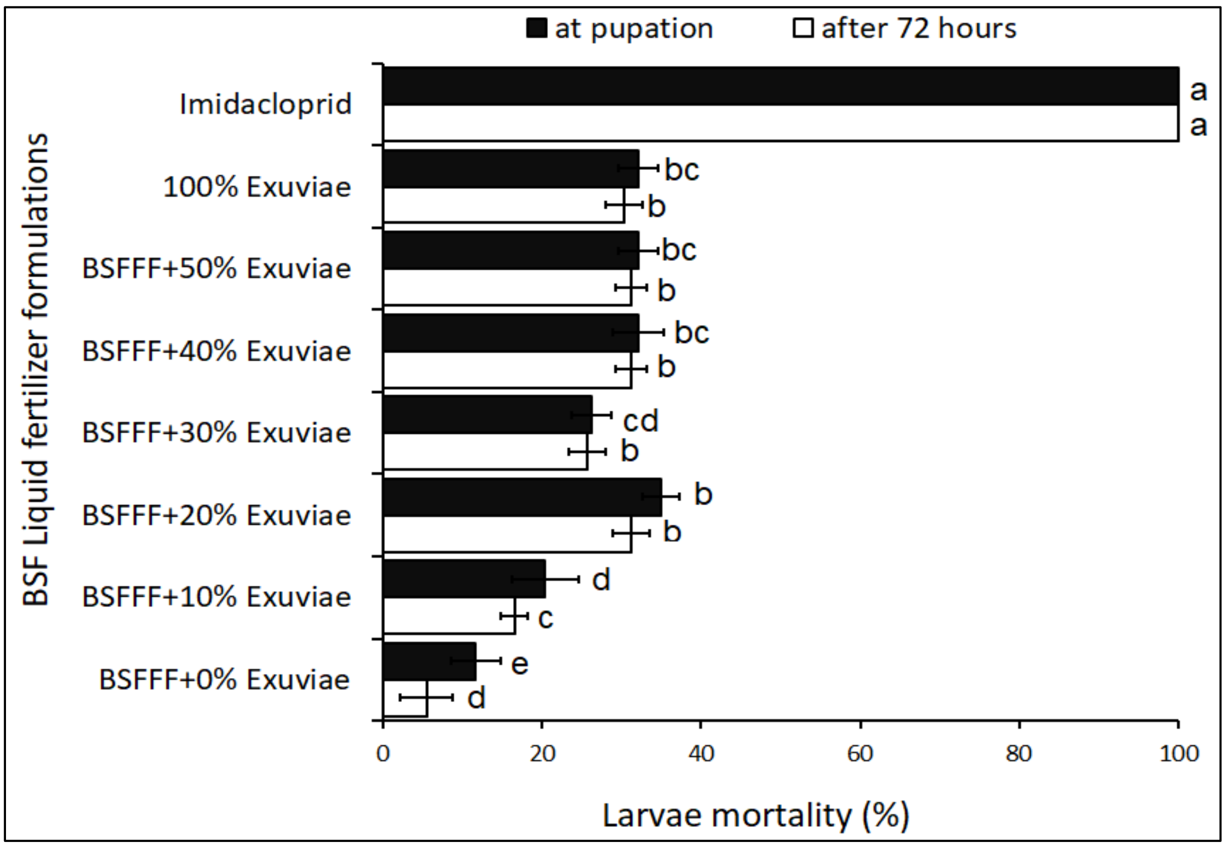

Figure 4.2: Mortality of first instar larvae of onion fly after 72 hours and at pupation

Note: Error bars represent standard errors. Error bars followed by the same letter(s) indicate no significant difference in larvae mortality at $p \leq 0.05$ based on GLM and Fisher's LSD p-value adjustment. The negative control was corrected using Abbot's equation (Abbott, 1925)

4.4.2.2 Mortality of second instar larvae of onion fly after residual exposure to BSF liquid fertilizer

The BSF liquid fertilizer had a significant effect on the mortality of second instar larvae of onion fly after residual exposure ($p < 0.05$). The highest mortality was recorded in formulations with 100% pupae exuviae, which was 10.9% after 24 hours, but slightly increased to 17.8% and 22.4% after 48 and 72 hours of exposure, respectively; however, this was not statistically different across all formulations with ≥ 30% pupae exuviae (Table 4.4). Liquid fertilizer formulations without pupae exuviae had the lowest overall mortality of 3.35% across all exposure periods (Table 4.4). The mortality of second-instar onion fly larvae was strongly correlated with the rates of pupae exuviae in black soldier fly liquid fertilizer formulations ($r = 0.90$, $p = 0.006$). Varying the level of BSF exuviae in liquid fertilizer formulations significantly affected the mortality rate of second instar larvae of onion fly (GLM: $\chi2 < 0.001$, df = 40, $p < 0.05$). Imidacloprid had the highest mortality of 98.3 % after 48 hours, which did not change significantly after 72 hours of exposure ($p = 0.191$), whereas negligible mortalities were recorded in the negative control.

Table 4.4: Mortality of second instar larvae of onion fly after 24, 48 and 72 hours of exposure by residual method

Formulation of BSF liquid fertilizer	Exposure time		
	24hrs	48hrs	72hrs
BSFFF+0% Exuviae	1.68e	2.54f	3.35f
BSFFF+10% Exuviae	4.20de	7.63e	9.48e
BSFFF+20% Exuviae	5.88cde	10.17de	12.07de
BSFFF+30% Exuviae	6.72bcd	12.71cd	16.38cd
BSFFF+40% Exuviae	8.40bcd	11.86cde	15.52cd
BSFFF+50% Exuviae	9.24bc	15.25bc	18.97bc
100% Exuviae	10.92b	17.80b	22.41b
Imidacloprid	80.67a	98.31a	99.83a
P	<0.05	<0.05	<0.05
LSD	4.49	4.47	5.43
CV%	24.12	17.37	18.79

Means followed by the same letter(s) in each column are not significantly different at p≤0.05; CV=Coefficient of Variation; LSD=Least Significant Difference at (p≤0.05); BSFFF – Black soldier fly frass fertilizer

Note: The negative control was corrected using Abbot's equation (Abbott, 1925)

4.4.2.3 Period until pupation of onion fly after residual exposure to BSF liquid fertilizer at first larval instar

Although, BSF liquid fertilizer with 20% exuviae rates delayed the pupation of onion fly by two days after residual exposure at neonate stage, varying the level of BSF exuviae in liquid fertilizer formulations did not significantly affect the time until the pupation of *A. orientalis* (GLM: $\chi 2$ = 0.185, df = 19, p = 0.201). Onion fly larvae subjected to liquid fertilizer formulations with 20-40% BSF exuviae took 13-15 days to pupate, whereas those subjected to all other formulations, including the control, pupated after 12 days (Figure 4.3). Increasing the rate of BSF exuviae in the liquid fertilizer beyond 40% did not affect the period until pupation (p = 0.250). *Atherigona orientalis* larvae subjected to imidacloprid at the first instar recorded 100% mortality after 72 hours; hence no survival was recorded at pupation.

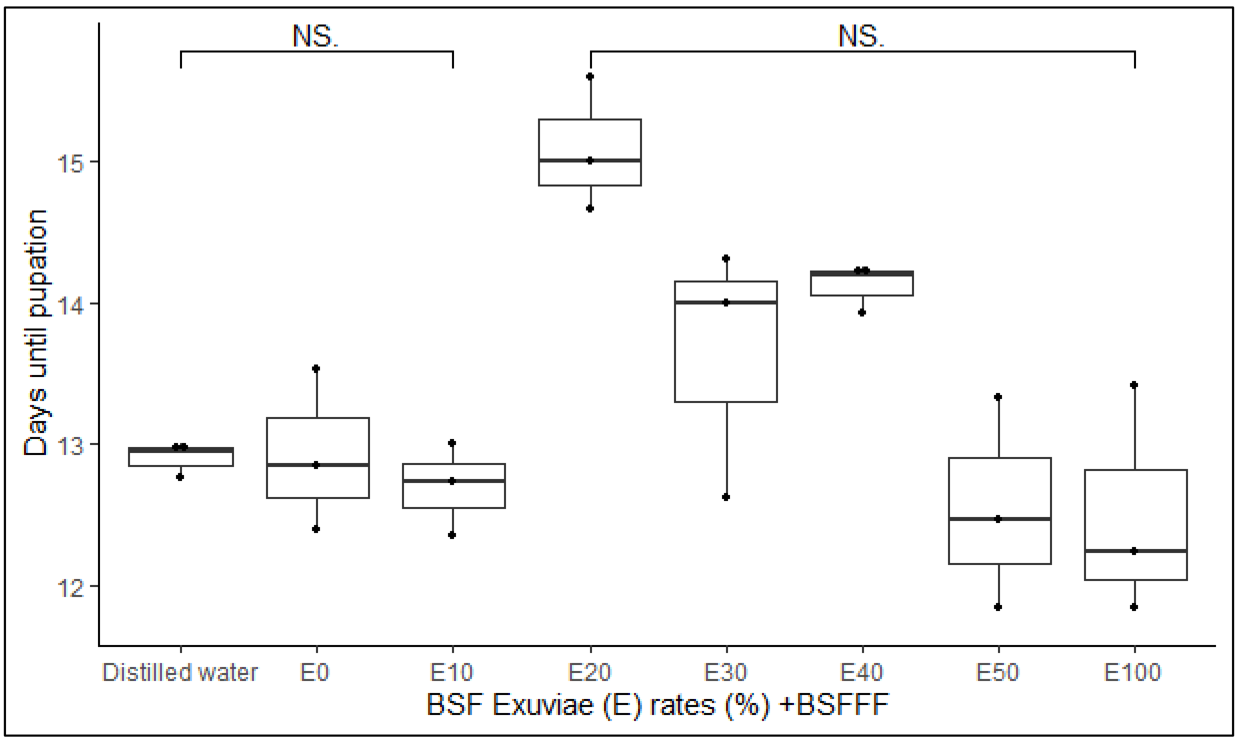

Figure 4.3: Number of days to pupation after residual exposure at 1st larval instar. The horizontal line within each box indicates the median and the bottom and top lines of the box indicate the 25th and 75th quartiles, respectively. The whiskers represent the smallest values within 25% quantiles - 1.5 × interquartile range (IQR) and the largest values within 75% quantiles +1.5 × IQR. Asterisk NS denotes "not significant"

4.4.3 Ovicidal activity of BSF liquid fertilizer against *Atherigona orientalis*

The black soldier fly liquid fertilizer significantly inhibited the survival of eggs of *A. orientalis* ($p < 0.05$). Black soldier fly liquid fertilizer formulations with ≥20% showed the highest egg mortality ranging from 60.4% to 64.9%, whereas formulations without pupae exuviae had the lowest mortality of 17.57% (Figure 4.4). There was a moderately positive correlation between egg mortalities and the level of pupae exuviae in the liquid fertilizer formulations ($r = 0.655$, $p = 0.111$). Increasing the level of BSF exuviae in the liquid fertilizer formulation resulted in a significant positive effect on egg mortality (GLM: $\chi2 = 0.0003$, df = 40, $p = 0.0007$). Imidacloprid had the highest impact on egg survival, with about 95.5% mortality, which was significantly higher than the maximum mortality recorded from liquid fertilizer formulations ($p < 0.05$).

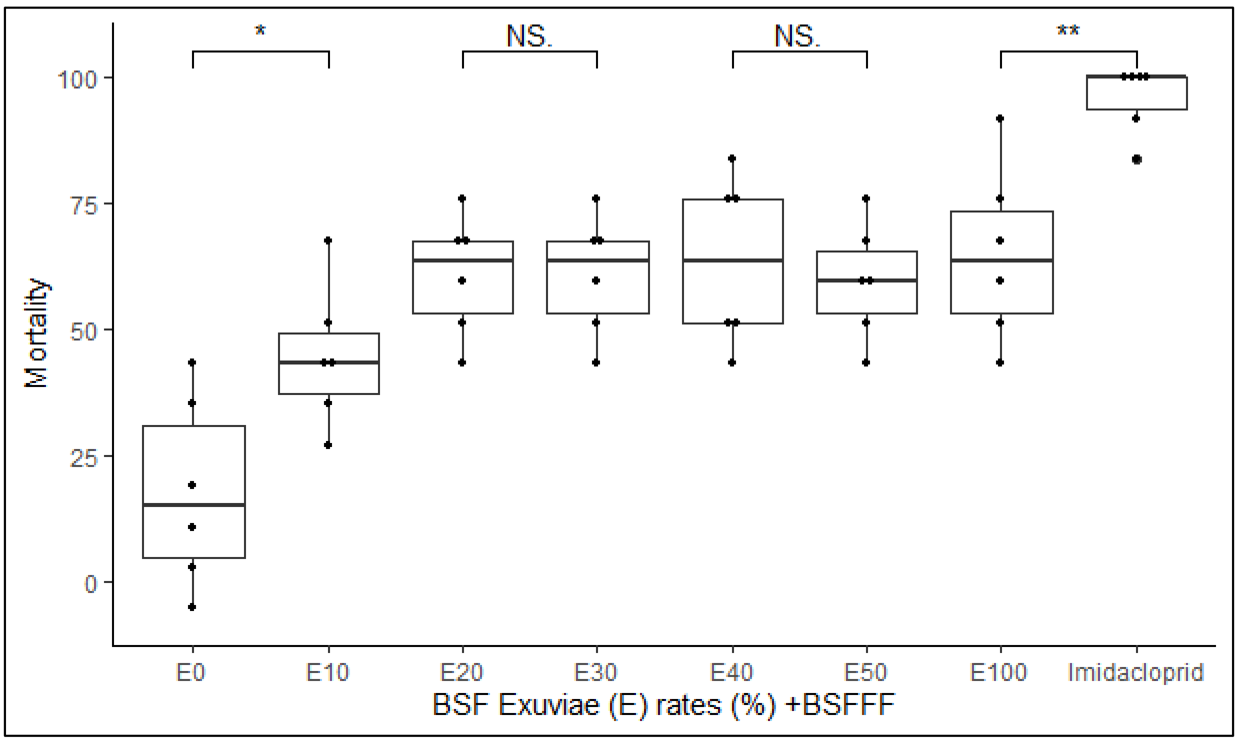

Figure 4.4: Mortality of *A. Atherigona* eggs 72 hrs after exposure to BSF liquid fertilizer formulations. The horizontal line within each box indicates the median and the bottom and top lines of the box indicate the 25th and 75th quartiles, respectively. The whiskers represent the smallest values within 25% quantiles - 1.5 × interquartile range (IQR) and the largest values within 75% quantiles +1.5 × IQR. Asterisks: ***, ** and * denotes the statistical significance of variables at 0.001, 0.01 and 0.05 p levels, respectively

Note: The negative control was corrected using Abbot's equation (Abbott, 1925)

4.5 Discussion

This study revealed significant mortality of *A. orientalis* eggs due to the activity of black soldier fly-liquid fertilizer. Black soldier fly liquid fertilizer with ≥20% pupae exuviae rates caused 65% mortality of *A. orientalis* eggs after contact exposure. Although there is limited study about the *A. orientalis*, amending the soil with black soldier fly frass fertilizer and pupae exuviae has revealed high mortality of *Delia* species. For instance, a recent study by Wantulla *et al.* (2022) reported a reduction in the survival of *Delia radicum* when soils were amended with black soldier fly frass and pupae exuviae. The high egg mortality can be attributed to the chitin in BSF pupae, which promotes the population of chitinolytic microorganisms, such as the lactic acid bacteria, Bacillus, *Pseudomonas* spp., *Flavobacterium* spp. and *Saccharomyces* spp. that digests the polysaccharide chitin to disaccharide chitobiose, thus degrading the eggshells and cell wall of the exposed juveniles (Kielak *et al.*, 2013; Brzezinska *et al.*, 2014; Zargar *et al.*, 2015; Chen & Peng, 2019). The activity of chitinolytic microorganisms on *A. orientalis* eggs could also be enhanced by the open meshwork of chorion covering the outer wall of the shells, which allows penetration by substances such as water, alcohol or relative mobile oils (Hinton & Cole, 1965); in this case, the chorion could be easily penetrated by the BSF liquid fertilizer establishing contact with the enclosed juvenile, hence inhibiting hatchability. Therefore, the study demonstrates that amending the soil with BSF frass fertilizer and at least 20% BSF pupae exuviae is sufficient to achieve the optimum effect on *A. orientalis* eggs.

The study also recorded a significant increase in mortality of the first and second instar larvae of the onion fly (*A. orientalis*) due to an increase in the proportion of pupae exuviae in the liquid fertilizer. The liquid fertilizer inhibited the survival of first and second instar larvae of onion fly by 31.2% and 22.4%, respectively, after residual exposure. Previous studies have also reported high mortality rates for neonates compared to second and third larval stages of insects from different families when subjected to compounds with insecticidal properties. For instance, young larval instars of *Musa domestica* and *Spodoptera littoralis* were found to be more sensitive to diflubenzuron than older ones (Grosscurt, 1978). Similarly, Seljåsen & Meadow (2006) reported significantly higher mortality of the first instar of *Mamestra brassicae* treated with neem extracts compared to second instar larvae exposed to similar conditions. First instar larvae are more vulnerable to abiotic and biotic stress, making them easy to control in IPM. Onion fly neonates emerge from eggs at the base of onion plants and are considered the most critical infestation stage. The low mortality of the second instar larvae may be attributed to the hardened cuticle, which may be tolerant to the effect of chitinolytic bacteria. Indeed cuticle hardness and sclerotization have been mentioned as a key parameters that may affect fungal and bacterial penetration during insect-pathogen interactions (Ekesi *et al.*, 2002; Myrand *et al.*, 2015). Therefore, applying BSF liquid fertilizer early in the season may prevent *A. orientalis* egg eclosion and reduce the survival of the neonates. Since BSF liquid fertilizer has a high nutrient content, its application can be synchronized with onion phenology to provide nutrients and protection at critical stages of crop development.

The high and moderate mortality recorded on eggs and larval stages of *A. orientalis* can be attributed to the abundance of chitin in BSF pupae exuviae, which possess insecticidal activities against insects from diverse families. According to previous studies, about 10.18% and 11.85% of chitin can be obtained from BSF pupae exuviae through chemical and biological extraction methods, respectively (Lagat *et al.*, 2021). These are linear polymers of β(1-4) linked N-acetyl-D-glucosamine and D-glucosamine that are the most abundant natural carbohydrate or amino polysaccharide polymers (Dutta *et al.*, 2004; Lin *et al.*, 2021). N-deacetylation of chitin can result in chitosan, which is a purer compound with high insecticidal activity. Previous observations have reported high toxicity of chitosan against insects from diverse families, such as *Spodoptera littoralis, Helicoverpa armigera, Plutella xylostella, Rhopalosiphum padi, Sitobion avenae, Metopolophium dirhodum, Myzus persicae, Hyalopterus prun*, and *Aphis gossypii* (Zhang *et al.*, 2003; Rabea *et al.*, 2005). The present study confirms that chitin-fortified BSF liquid fertilizer possesses high and moderate insecticidal activity against *A. orientalis* eggs and larvae, respectively. The low mortality recorded against the second instar larvae in both contact and residual assays can be attributed to a reduction in susceptibility that increases as the insect grows.

Although the difference was not statistically significant, the study also revealed a delay in the pupation of onion flies by two to three days after residual exposure to black soldier fly-liquid fertilizer at the neonate stage. The possible explanation for this result is that, due to residues of chitin in treated onion paste, the surviving *A. orientalis* larvae experienced reduced feeding and low biomass, which affected their development rate. Previous studies have also reported an increase in time until the eclosion of cabbage root fly subjected to soils amended with BSF frass and pupae exuviae (Wantulla *et al.*, 2022). Moreover, chitin residues in the liquid fertilizer could have interfered with the moulting process of the larvae at subsequent instars delaying pupation. According to previous studies, chitin and chitosan can disrupt the ecdysis process by interfering with the ecdysone hormone, thus inhibiting moulting (Rabea *et al.*, 2005). Nevertheless, formulations with 100% pupae exuviae did not increase the larval period until pupation; instead, they exhibited a slight reduction in the days to pupation compared to the control. These results can be attributed to a possible abundance of beneficial bacteria in liquid fertilizer made from 100% pupae exuviae. According to previous observations, inoculation of soil with bacteria, such as rhizobacteria (*Pseudomonas* spp.), which induce systemic resistance in plants, can also enhance the performance of root flies, such as *D. radicum* (Friman *et al.*, 2021). Nonetheless, further evaluation should be conducted to characterize the microbial community in the liquid fertilizer and determine their interaction with onion flies.

Therefore, black soldier fly liquid fertilizer applications should target eggs and neonates of the first generation early in the season. The study recommends a drenching approach to increase the chances of contact between the liquid fertilizer and the eggs laid at the base of the stem. However, a field experiment should be conducted to confirm the results presented in this study. Chitosan may also elicit the production of secondary metabolites as a defence against herbivorous pests, which may alter the insects' olfactory cues, repelling them or attracting their natural enemies, thus controlling them (Orlita *et al.*, 2008; Salimgandomi & Shabrangi, 2016; Fernández *et al.*, 2020). Therefore, further studies should assess the effect of chitin-fortified BSF liquid fertilizer on the defence mechanisms of crops and the abundance of root flies' natural enemies in field conditions. Nevertheless, chitin-fortified BSF liquid fertilizer could be integrated into IPM for the effective management of onion fly infestation.

4.6 Conclusion

The present study demonstrates that black soldier fly liquid fertilizer can inhibit the survival of *A. orientalis* eggs and larvae. Liquid fertilizer with ≥20% pupae exuviae had the highest effect on eggs of *A. orientalis,* with mortality ranges of 60-65%. The BSF liquid fertilizer also reduced the survival of the first and second instar larvae of onion fly by 31.2% and 22.4%, respectively. The egg and larval mortality of *A. orientalis* can be attributed to the abundance of chitinolytic microorganisms in the BSF liquid fertilizer formulations, which can degrade the shells and cuticles of eggs and larvae by digesting the polysaccharide chitin to disaccharide chitobiose. The delay in the period until pupation of onion fly by two to three days can be attributed to a possible reduction in feeding, affecting the development rate and probable interference with the ecdysis process. The study proposes BSF liquid fertilizer as a viable candidate for integrated pest management of onion fly which can be applied early in the season through soil drenches to offer nutrients and protection against the first generation of onion fly. Nevertheless, further research is required to confirm the feasibility of liquid fertilizer in field conditions as well as against other root flies in the genus *Delia*.

CHAPTER FIVE
EFFECT OF FORTIFIED BLACK SOLDIER FLY FRASS FERTILIZER ON AGRONOMIC PERFORMANCE OF ONION (*Alium cepa* L.)

5.1 Abstract

High costs of commercial fertilizer and pesticides have become a major concern among vegetable farmers, calling for sustainable and affordable alternatives. Although waste streams from black soldier fly mass rearing have the potential to improve crop growth and yield, the optimum amendment rate of frass fertilizer and pupae exuviae, and the actual effect on onion yield are yet to be established. This study determined the effect of amending soil with different rates of black soldier fly frass fertilizer and chitin-rich pupae exuviae on onion crop growth and yield. Black soldier fly frass fertilizer was applied at a constant rate of 4.12 t ha^{-1}, while pupae exuviae rates were 0 t ha^{-1}, 0.41 t ha^{-1}, 0.81 t ha^{-1}, 1.65 t ha^{-1}, 2.43 t ha^{-1} and 3.24 t ha^{-1}, based on chitin content. Control plots were treated with 200 Kg ha^{-1} di-ammonium phosphate at planting and 300 kg ha^{-1} calcium ammonium nitrate after six weeks. Plant growth was assessed every three weeks, and mature onion bulbs were harvested at commercial maturity. Plots amended with 8% and 6% chitin had the highest number of leaves and plant height, which was significantly higher than commercial fertilizers. Amending the soil with 8% chitin increased onion bulb weight and diameter by 44.5% and 23.5%, respectively, which was significantly higher than the commercial fertilizers. Nonetheless, applying 8% chitin increased the total biomass, total yield, marketable yield and shoots biomass by 46.9%, 50.5%, 49.6% and 35.8%, respectively, but this was statistically similar across all treatments with chitin. Therefore, the study recommends soil amendment with black soldier fly frass fertilizer and pupae exuviae as viable substitutes to commercial fertilizers with additional benefits such as enhanced biodiversity, environmental friendliness and promotion of crop health.

Keywords: Black soldier fly, frass fertilizer, pupae exuviae, chitin, and onions

5.2 Introduction

The mass rearing of black soldier fly (*Hermetia illucens*) for valorisation of organic wastes and provision of nutrient-rich livestock feed has resulted in an abundance of insect residue streams which can be leveraged to enhance soil fertility in crop production. Black soldier fly larvae have the ability to convert organic wastes into protein-rich livestock feed and organic manure (Lalander *et al.*, 2015; Oonincx *et al.*, 2015; Beesigamukama *et al.*, 2020; Schmitt & de Vries, 2020; Torgerson *et al.*, 2021; Barragán-Fonseca *et al.*, 2022;). The waste streams from BSF rearing comprise frass and pupae exuviae which are rich in plant nutrients and chitin that possess pesticidal properties (Houben *et al.*, 2020). Amending the soil with chitin-rich arthropod shells has been reported to stimulate the activity of beneficial microbes, supply plant nutrients, and enhance plant growth and yield. Although numerous studies have focused on soil amendments using crustacean shells, these products are limited and unavailable to farmers in sub-Saharan Africa. With the recent adoption of BSF farming, chitin-rich pupae exuviae have become more abundant, hence the need to utilize these waste streams to enhance crop production. However, the optimum amendment rates of BSF frass fertilizer and chitin-rich pupae exuviae still need to be discovered. Therefore, the current study sought to determine the effect of amending soil with BSF frass fertilizer and pupae exuviae on onion crop growth and yield. The study also aimed to determine the optimal amendment rates of BSF frass fertilizer and pupae exuviae to avoid wastage or any potential negative consequences of insufficient or excessive application.

5.3 Materials and Methods

5.3.1 Description of the experimental site

The field trial was conducted at the International Centre for Insect Physiology and Ecology (ICIPE), Duduville Campus, in Nairobi (1.2194° S, 36.8915° E). The elevation is 1600 m above sea level in the upper semi-humid agroecological zone (Jaetzold & Schmidt, 1983). The region experiences a bimodal rainfall distribution, with the long rain season lasting from mid-March to mid-May and the short rain season from mid-October to mid-December, with average precipitation of 409 and 220 mm, respectively (Jaetzold & Schmidt, 1983). The study was conducted in a field condition between October 2022 and January 2023 under supplemental irrigation.

5.3.2 Source and preparation of black soldier fly pupae exuviae

The BSF pupae exuviae were obtained from the stock colony at *icipe*. The insect underwent six instars of larval development and emerged into pupae after 13-16 days. The pupae were collected and placed in the adult fly cages for moulting, followed by the collection of pupae exuviae. The shells were carefully sorted to remove substrate wastes, followed by soaking in cold water for 12 hours before washing and sun drying for four days. The dry shells were ground into a fine powder using an electronic grinder to increase the surface area for microbial action.

5.3.3 Preparation of black soldier fly frass fertilizer

The black soldier fly larvae were reared on spent grain and potato waste according to the procedure described by Shumo *et al.* (2019). The larvae were harvested after two weeks leaving behind the frass fertilizer, which was collected and composted for five weeks following the procedure outlined by Beesigamukama *et al.* (2020). A heap of 1 m width, 2 m length and 1 m height, was established on a flat soil surface lined with a polythene sheet. The heap was hydrated to 55-65% moisture content, as described by Chen *et al.* (2011) and covered with a black polythene sheet to prevent heat loss and maintain optimum moisture for microbial action. The temperature was monitored and the composted materials were turned every two weeks using a spade to enhance aeration. After two months, the compost frass fertilizer was harvested, and the nutrient profile was determined to establish the rates of different macro and micro nutrients before application (Table 5.4).

5.3.4 Preparation of onion seedlings

The onion variety Malbec F1 was selected due to its early maturity, high yield potential, and wide adoption by farmers. Seeds were obtained from Greenlife Crop Protection Africa Limited and propagated in plastic seedling trays for six weeks before transplanting at the 3-5 leaf stage. The nursery soil was mixed with well-decomposed BSF frass fertilizer before sowing. Watering, shading, weeding and rogueing were done regularly to obtain high-quality seedlings.

5.3.5 Description of treatments, experimental design and layout

The study involved six treatments where plots were amended with different rates of BSF frass fertilizer (BSFFF) and chitin, a positive control (DAP 200 kg ha^{-1} and CAN 300 kg ha^{-1}), and negative control (with no fertilizer). The BSFFF rate of 4.12 t ha^{-1} was calculated based on N requirements of onion – 120 kg N ha^{-1} and was constant across the six treatments (Khokhar, 2019; Jilani *et al.*, 2004; Nasreen *et al.*, 2008), whereas different rates of pupae exuviae were applied based on chitin levels as a percentage of BSFFF rates: 0%, 1%, 2%, 4%, 6%, and 8% chitin levels. Black soldier fly exuviae has 10.18% chitin (Lagat *et al.*, 2021), and these rates were used to calculate the level of chitin in each treatment. In this context, the pupae exuviae levels were: 0 g (0 t ha^{-1}), 162.03 g (0.41 t ha^{-1}), 324.06 g (0.81 t ha^{-1}), 658.12 g (1.65 t ha^{-1}), 972.18 g (2.43 t ha^{-1}), and 1296.24 g (3.24 t ha^{-1}), respectively. The BSFFF and chitin were incorporated into the soil seven days before planting day to allow sufficient time for the breakdown of the chitin, whereas DAP 200 kg ha^{-1} (18% N and 46% P_2O_5) was applied as a positive control at planting. The positive control was top-dressed by CAN at a rate of 300 kg ha^{-1} six weeks after transplanting. The field experiment was conducted during the September to January season with supplemental irrigation. Onion seedlings were transplanted into plots measuring 2 × 2 m at a spacing of 30 × 10 cm and a cropping density of 134 plants per plot. Randomized Complete Block Design (RCBD) with three replications was adopted with an inter-plot and inter-block spacing of 0.5 m and 1 m, respectively. The crops were maintained free of weeds through weeding and rogueing, whereas imidacloprid solution (6 mL/L) was applied to control thrips spotted six weeks after transplanting.

5.3.6 Assessment of plant growth and yield

The parameters assessed in this study included plant height, number of leaves, total biomass, shoot biomass, total yield, marketable yield, bulb weight and bulb diameter (Gateri *et al.*, 2018). Fifteen plants from the five innermost rows were selected for growth assessment. Evaluation of plant height and number of leaves was done three weeks after transplanting, and subsequent assessment was done every three weeks until harvest. Plant height was measured in centimetres from the ground level up to the highest leaf using a tape measure while the number of leaves was determined by counting the leaves capable of photosynthesis (> 5 cm in height) from the 15 plants in the sampling frame. The percentage of fallen plants at maturity was evaluated by visually counting plants with fallen tops in each plot. Onion plants were harvested after 50% of the plants had fallen over (Gateri *et al.*, 2018). After harvesting, the shoot was carefully detached from the bulb, and the fresh weight of the sample was computed in kilograms using an electronic weighing scale. The total yield was determined by measuring the weight of bulbs (in kgs) in each plot using an electronic weighing scale, whereas the marketable yield was determined by excluding bulbs < 20 mm in diameter as well as sprouted, rotten, bolted and split bulbs. Plant yield and biomass were expressed in t ha^{-1} using the following equation.

$$\text{Yield (t ha}^{-1}) = \frac{(W \times 10{,}000)/A}{1000}$$

Where W is the total weight (in kgs) of harvested bulbs per plot, and A is the plot size in m^2.

The average weight of a single bulb was determined by dividing the total weight of bulbs in each plot by the number of bulbs and recorded in grams. The average bulb diameter was determined from thirty bulbs that were randomly sampled from each plot; using a Vernier calliper the diameter was taken at the widest circumference of the bulb and at a right angle to the longitudinal axis and recorded in mm.

5.3.7 Data analysis

The data were subjected to Analysis of Variance (ANOVA) using R. statistical software (R Core Team, 2022) followed by mean separation using Fisher's Least Significant Difference (LSD) test at a 5% probability level of significance. Data normality was assessed using the Shapiro-Wilk test. The yield was also analysed using generalized linear models (GLM) with Poisson models.

5.4 Results

5.4.1 Effect of soil amendments on the number of onion leaves

BSF exuviae rates had a significant effect on the number of onion leaves at different stages except during the first three weeks (Table 5.1). The average number of leaves increased from three at the transplanting stage to nine at physiological maturity and later reduced to eight at commercial maturity. Among the different amendment rates, BSF frass fertilizer amended with 8% and 6% chitin attained the highest number of leaves at physiological maturity, which was significantly higher than the commercial fertilizers and the control ($p = 0.037$). Although each plot exhibited a reduction in the number of leaves at commercial maturity, plots with 8% and 6 % chitin still had the highest number of leaves ($p = 0.018$) (Figure 5.1).

Table 5.1: Effect of black soldier fly (BSF) frass fertilizer and chitin-rich pupae exuviae on the number of onion leaves

Amendment rates	Duration of onion growth				
	3 weeks	6 weeks	9 weeks	12 weeks	15 weeks
BSFFF+0% Chitin	3.13b	5.38c	7.24bc	8.38cde	8.09abc
BSFFF+1% Chitin	3.16b	5.71ab	7.53ab	8.84abc	8.03bc
BSFFF+2% Chitin	3.49a	5.89a	7.31abc	8.67bc	7.69cde
BSFFF+4% Chitin	3.38a	5.71ab	7.29abc	8.44cd	7.47de
BSFFF+6% Chitin	3.42a	5.87a	7.38abc	9.09ab	8.42ab
BSFFF+8% Chitin	3.38a	5.93a	7.62a	9.22a	8.49a
DAP	3.44a	5.60bc	7.13c	8.27de	7.91cd
Control	3.13b	5.38c	6.67d	7.89e	7.27e
Mean	3.32	5.68	7.27	8.60	7.92
p	0.062	0.017	0.040	0.037	0.018
LSD	0.19	0.23	0.34	0.51	0.45
CV%	4.97	3.54	4.11	5.26	5.04

Means followed by the same letter(s) in each column are not significantly different at $p \leq 0.05$; CV%=Coefficient of Variation; LSD=Least Significant Difference at ($p \leq 0.05$). BSFFF – Black soldier fly frass fertilizer

5.4.2 Effect of soil amendments on onion plant height

Different amendment rates of black solder fly frass fertilizer and chitin-rich pupae exuviae significantly affected the plant height from seedling stage to physiological maturity (12 weeks) ($p < 0.05$). After the 12th week, the difference in plant height across different treatments was insignificant ($p = 0.675$). The average plant height increased significantly from 10 cm at the transplanting stage to 58.8 and 58.07 cm at physiological and commercial maturity (Table 5.2). Plots amended with commercial fertilizers had the highest plant height in the first three weeks after transplanting (p= 0.029), after which plots amended with chitin took over attaining the highest height. Plots amended with 8% chitin which was 0.33 ta ha^{-1}, attained the highest average height of 62 cm at physiological maturity, but this was statistically similar across all plots amended with BSF chitin (Table 5.2). Plots amended with BSF frass fertilizer alone had a comparable height with those amended with commercial fertilizers at physiological and commercial maturities (Table 5.2).

Table 5.2: Effect of BSF frass fertilizer and chitin-rich pupae exuviae on onion plant height

Amendment rates	Duration of plant growth (height in cm)				
	3 weeks	6 weeks	9 weeks	12 weeks	15 weeks
BSFFF+0% Chitin	14.20cd	32.89c	50.10bc	55.85cd	56.10b
BSFFF+1% Chitin	14.22cd	36.60ab	52.42ab	58.32abcd	57.80ab
BSFFF+2% Chitin	14.93bc	36.41ab	51.70abc	59.37abc	57.37ab
BSFFF+4% Chitin	14.90bc	35.89b	52.24ab	61.43a	58.96ab
BSFFF+6% Chitin	16.27ab	37.47ab	52.33ab	60.40ab	59.56ab
BSFFF+8% Chitin	16.58a	38.36a	54.42a	61.75a	61.15a
DAP	16.92a	36.24b	49.71bc	57.61bcd	58.54ab
Control	12.93d	31.76c	48.46c	55.64d	55.06b
Mean	15.12	35.70	51.42	58.80	58.07
p	0.029	0.004	0.039	0.021	0.675
LSD	1.52	1.99	3.48	3.68	4.56
CV%	8.93	4.93	5.97	5.53	6.92

Means followed by the same letter(s) in each column are not significantly different at $p \leq 0.05$; CV%=Coefficient of Variation; LSD=Least Significant Difference at ($p \leq 0.05$). BSFFF – Black soldier fly frass fertilizer.

5.4.3 Effect of soil amendments on the total and shoot biomass

Amending the soil with different rates of black solder fly frass fertilizer and chitin-rich pupae exuviae significantly affected total plant biomass (p = 0.047). Applying 8% chitin resulted in the highest total and shoot biomass increase of 46.9% and 35.8%, respectively (Table 5.3). Plots amended with BSF frass fertilizer without chitin had a 19.2% and 5.1% increase in total and shoot biomass, which was comparable to commercial fertilizers (Table 5.3). Among the different amendment rates, plots amended with 8% BSF chitin had the highest total crop biomass and shoot biomass of 66.2 t ha^{-1} and 15.1 t ha^{-1}, respectively, which was however statistically similar across all treatments with chitin (p = 0.769). Increasing the rate of chitin did not significantly increase the total biomass (GLM: df =16, p = 0.06). Plots amended with BSF frass fertilizer without chitin had the lowest total and shoot biomass, which was comparable to rates recorded in plots with commercial fertilizers as well as the control (Table 5.3).

5.4.4 Effect of soil amendments on total and marketable yields

Increasing the rate of chitin in soil amendments from 0 to 8% significantly increased the total biomass, total yield and marketable yield ($p < 0.05$) (Table 5.3). Plots amended with 8% chitin recorded the highest increase in the total and marketable yields by 50.5% and 49.6%, respectively, which was significantly higher than those amended with the commercial fertilizers– 34.6 and 32.9%, respectively (Table 5.3). Among the different amendment rates of BSF frass fertilizer and chitin-rich pupae exuviae, plots with 8% BSF chitin had the highest total and marketable yield of 51.10 t ha^{-1} and 50.60 t ha^{-1}, respectively, whereas those without BSF chitin had the lowest (Table 5.3). Plots with commercial fertilizers also recorded high total and marketable yields, which were comparable to yields from plots amended with 1 to 2% chitin as well as those amended with BSF frass fertilizer without chitin. Although increasing the rate of chitin from 1-8% increased overall yield, the change was not significant for total yield (GLM: $\chi2= 0.052$, df =16, p = 0.07) and marketable yield (GLM: $\chi2= 0.056$, df =16, p = 0.074).

Table 5.3: Effect of BSF frass fertilizer and chitin-rich pupae exuviae on onion yields

Amendment rates	Yield (t ha^{-1})			
	Total biomass	Total yield	Marketable yield	Shoot biomass
BSFFF+0% Chitin	53.75c	42.05b	41.94b	11.70b
BSFFF+1% Chitin	58.79abc	45.39ab	45.39ab	13.40ab
BSFFF+2% Chitin	61.61ab	47.98ab	47.43ab	13.63ab
BSFFF+4% Chitin	62.84ab	49.0a	49.19a	13.76ab
BSFFF+6% Chitin	61.10abc	48.32ab	48.18ab	12.78ab
BSFFF+8% Chitin	66.22a	51.10a	50.60a	15.12a
DAP	57.83bc	45.71ab	44.96ab	12.11ab
Control	45.08d	33.95c	33.83c	11.13b
Mean	58.40	45.45	45.19	12.95
p	0.047	0.045	0.049	0.769
LSD	7.79	6.59	6.58	3.35
CV%	11.77	12.80	12.86	22.83

Means followed by the same letter(s) in each column are not significantly different at $p\leq0.05$; CV%=Coefficient of Variation; LSD=Least Significant Difference at ($p\leq0.05$). BSFFF – Black soldier fly frass fertilizer

5.4.4 Effect of soil amendments on mean onion bulb weight

The average bulb weight significantly increased with an increase in chitin rates ($p = 0.045$). Applying BSF frass fertilizer with 8% chitin increased the bulb weight by 44.5% compared to a 34.6% increase by commercial fertilizers (Figure 5.1). Plots amended with 8% BSF chitin had the highest bulb weight of 0.153 kg, but this did not significantly vary across all plots amended with chitin. Applying BSF frass fertilizer without chitin resulted in the lowest yield, which was, however, statistically similar to that in plots amended with commercial fertilizers (Figure 5.1). Nevertheless, increasing the rate of chitin in soil amendments did not significantly influence average bulb weight (GLM: $\chi2= 0.052$, df =16, $p = 0.07$).

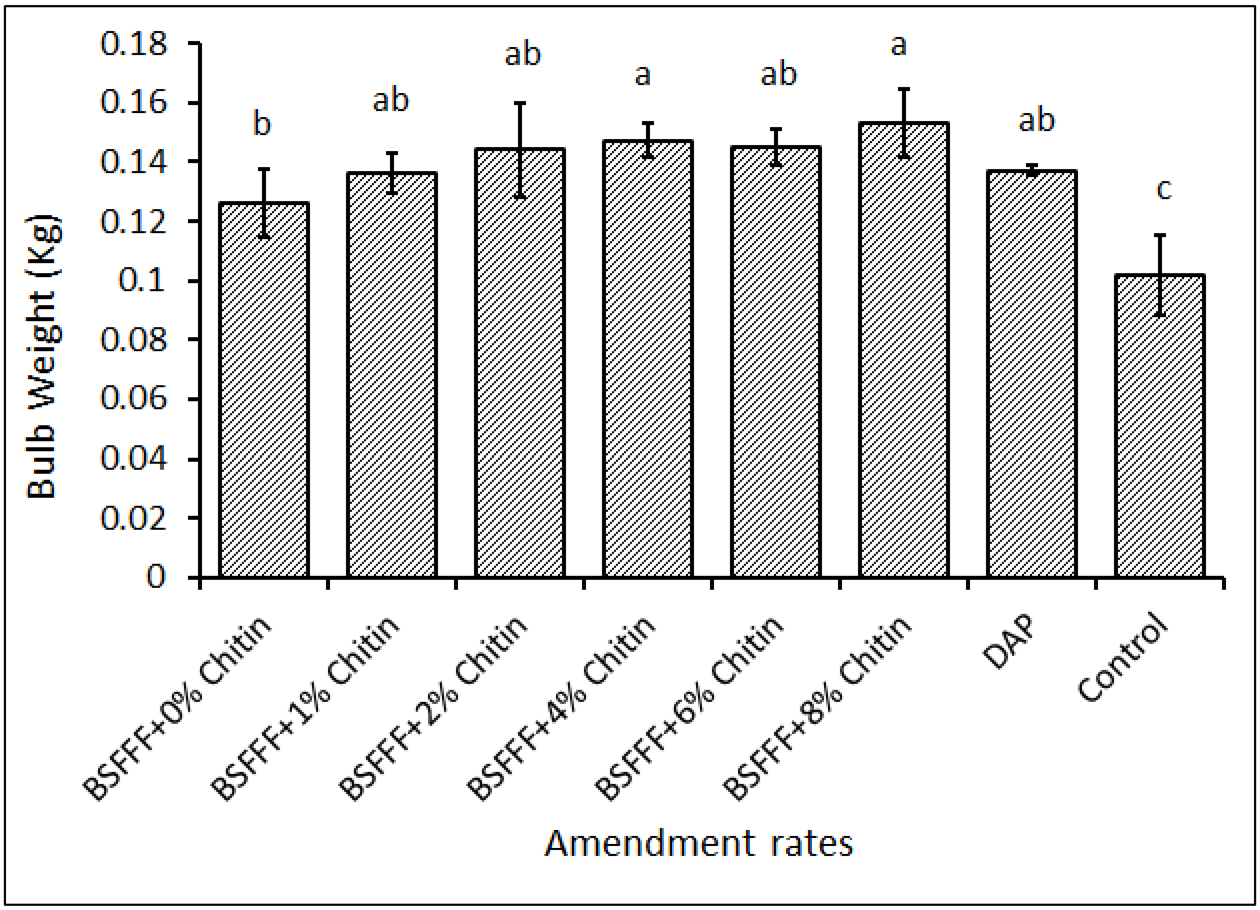

Figure 5.1: Effect of BSF frass fertilizer and chitin-rich pupae exuviae on onion bulb weight

Note: Error bars represent standard errors. Error bars followed by the same letter(s) indicate no significant difference in onion bulb weight at $p \leq 0.05$ based on Fisher's LSD p-value adjustment.

5.4.5 Effect of soil amendments on mean onion bulb diameter

Amending the soil with BSF frass fertilizer and chitin significantly increased the bulb diameter ($p = 0.0004$). Varying the rate of chitin from 1% to 8% increased the bulb diameter from 16.2 – 23.5%, whereas commercial fertilizers increased the bulb diameter by 16.1% (Figure 5.2). Plots amended with 4% and 8% BSF chitin had the highest bulb diameter of 75 and 78mm, respectively, whereas those amended with the commercial fertilizers and BSF frass fertilizer without exuviae, had the lowest bulb diameters of 73.0 and 73.1 mm, respectively (Figure 5.2). The negative control recorded the smallest onion bulbs with about 63 mm average diameter. Nevertheless, increasing BSF chitin rate in frass amendments from 1 to 8% did not significantly increase the average bulb diameter (GLM: $\chi2= 0.05$, df =16, $p = 0.068$).

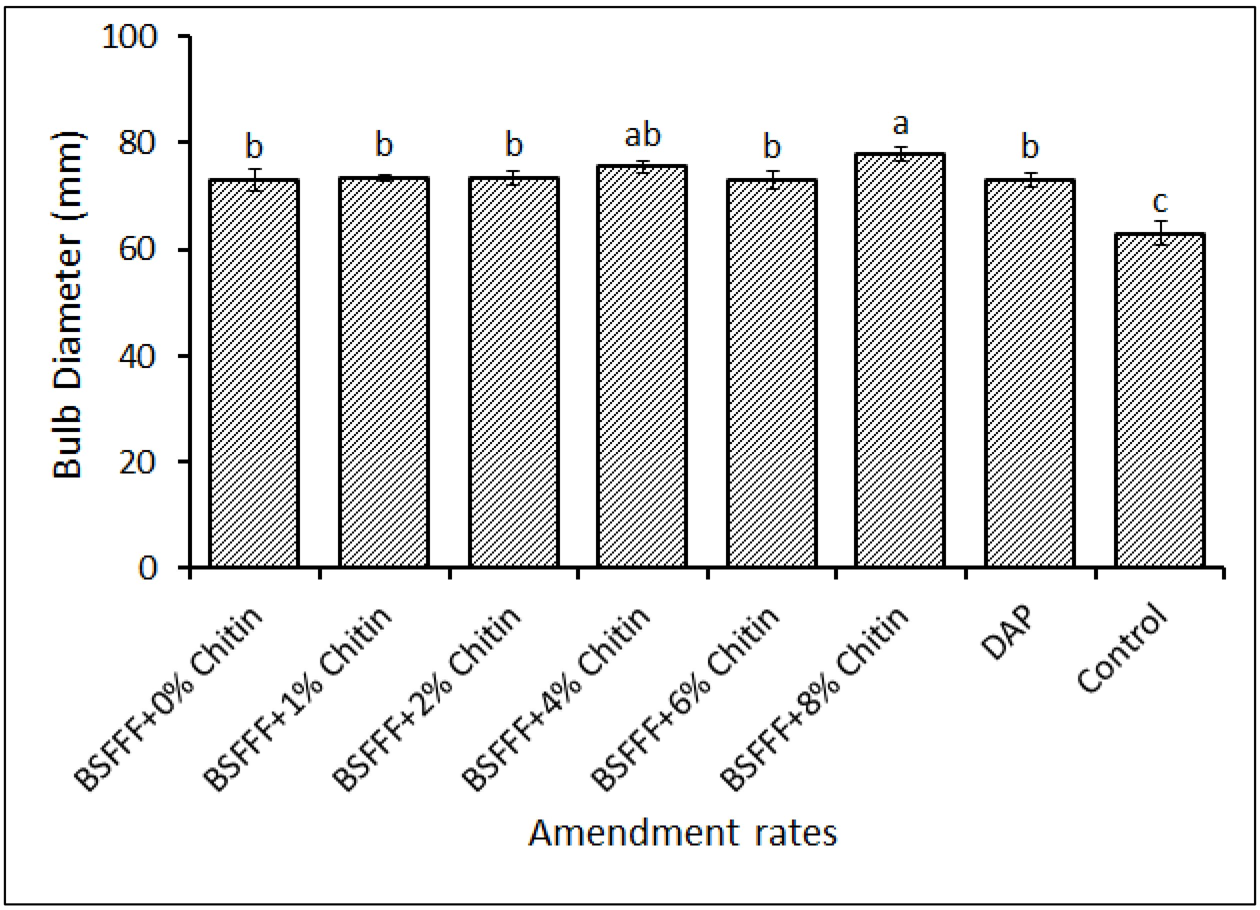

Figure 5.2: Effect of BSF frass fertilizer and chitin-rich pupae exuviae on onion bulb diameter

Note: Error bars represent standard errors. Error bars followed by the same letter(s) indicate no significant difference in onion bulb diameter at $p \leq 0.05$ based on Fisher's LSD p-value adjustment.

5.4.6 Elemental composition of black soldier fly frass fertilizer and pupae exuviae

Table 5.4 below presents the physical-chemical characteristics of the black soldier fly frass fertilizer and pupae exuviae used in the experiment. The elements were determined based on the dry weight of BSFFF and pupae exuviae.

Table 5.4: Nutrient profile of BSFFF and pupae exuviae

Parameter	**Frass fertilizer**	**Pupae exuviae**
pH	10.20	7.06
Electrical conductivity (S) (mS/cm)	25.23	3.79
Dry matter (%)	85.07	92.13
Organic carbon (%)	42.17	43.03
Nitrogen (%)	2.91	6.55
Phosphorus (%)	0.60	0.48
Potassium (%)	6.53	0.75
Calcium (%)	0.30	6.47
Magnesium (%)	0.37	0.41
Sulphur (%)	0.43	0.24
Manganese (ppm)	199.00	2856.67
Iron (ppm)	6546.67	2793.33
Zinc (ppm)	59.20	123.00
Copper (ppm)	22.90	10.21
Boron (ppm)	44.80	10.16
Sodium (ppm)	13900.00	1350.00
C: N ratio	14.53	6.58

(mS/cm) – Electrical conductivity in Milli Siemens per centimetre

5.5 Discussion

The study revealed a significant increase in onion growth due to black soldier fly frass fertilizer and chitin-rich pupae exuviae amendments. An increase in BSF chitin rates was associated with a corresponding increase in the number of leaves and plant height. An application of 4.12 t ha^{-1} BSF frass fertilizer and 8% chitin increased the plant height by 11.0% and leaf density by 16.9%, at physiological maturity. This can be attributed to high nitrogen rates in the black soldier fly pupae exuviae and frass fertilizer of 6.6% and 2.9%, respectively, which are vital for plant growth. The addition of chitin-rich BSF pupae exuviae may have provided additional N, which increased the growth of onion (Abdissa *et al.*, 2011). The number of leaves and plant height reported in this study is higher than in previous studies where a maximum height of 50.12 cm was achieved at 160 kg N ha^{-1} (Jilani *et al.*, 2004). Similarly, Gateri *et al.* (2018) reported maximum plant height and leaf density of 45.4 cm and 8.7, respectively, after applying 104 kg N ha^{-1}. Other studies have reported 73.2 cm plant height at 180-240 kg N ha^{-1} (Lee *et al.*, 2003), 61.2 cm at 200 kg N ha^{-1} (Rizk *et al.*, 2012) and 41.8 cm at 100 kg N ha^{-1} (Khan *et al.*, 2007). The significant increase in plant height by BSF frass fertilizer and pupae exuviae could be attributed to the synthesis of amino acids that make up the metabolic process required for plant growth. Nevertheless, chitin-rich BSF pupae exuviae may have also stimulated the abundance of beneficial indigenous microbes in the soil resulting in high growth and yield (Barragán-Fonseca *et al.*, 2022).

Although plots with commercial fertilizers reported the highest growth during the first three weeks, it was quickly overtaken by plots amended with BSF frass fertilizer and chitin-rich pupae exuviae after six weeks. The exceptional performance of commercial fertilizers during the first three weeks can be attributed to their quick release of nutrients compared to BSF frass fertilizer which has a rather high C:N ratio of 14.5. Black soldier fly pupae exuviae also has calcium carbonate, which might have impaired the quick release of nutrients during the first weeks of transplanting (Jilani *et al.*, 2004; Shamshina *et al.*, 2020). Nevertheless, the BSF frass and chitin amendment was done one week before transplanting to allow sufficient time for biodegradation and release of nutrients during the vegetative stage. In a previous study by Aklog *et al.* (2016), chitin-rich shrimp meal fertilizer performed worse than the commercial fertilizer (HYPONeX), primarily because the former was applied at transplanting and thus did not release sufficient nutrients during the critical stage of crop development. Therefore, BSF chitin-rich pupae should be applied at least one week before transplanting to allow sufficient decomposition and timely release of nutrients.

The study also presents black soldier fly frass fertilizer and chitin-rich exuviae as excellent candidates for controlled N release in the soil to match crops' demand and uptake pattern, especially in soils prone to nitrate leaching (Mogren *et al.*, 2008). Slow release of nutrients has reported high yield compared to soluble nitrogen fertilizer in wet years (Ristimäki & Papadopoulos, 2000; Ransom *et al.*, 2020). Amending BSF frass fertilizer with pupae exuviae could be a better option for achieving controlled release of N into the soil compared to other novel approaches, such as polymer coating, which may be sophisticated and expensive for local farmers in Sub-Saharan Africa (Adams *et al.*, 2013; Halvorson *et al.*, 2014). Slow and controlled release of nutrients to the soil using BSF frass fertilizer and chitin-rich pupae exuviae is also associated with increasing nitrogen use efficiency, reducing the frequency of nitrogen application, and reducing the rate of N lost to the environment. Nonetheless, further studies are required to determine the release rate of Nitrogen by BSF frass fertilizer and pupae exuviae in field conditions considering numerous intervening factors such as soil microbiomes, temperature and moisture content.

Soil amendment with black soldier fly frass fertilizer and chitin resulted in a significant increase in bulb diameter and mean bulb weight by 23.5% (78 mm) and 44.5% (153 g), respectively. These results correspond with previous studies, which reported mean bulb diameter and bulb weight of 73 mm and 145 g at 138 kg N ha^{-1} (Abdissa *et al.*, 2011) and 51.4 mm and 67.4 at 104 kg N ha^{-1} (Gateri *et al.*, 2018). Jilani *et al.* (2004) reported a maximum bulb diameter of 59 mm at 180 kg N ha^{-1}, whereas Ghaffoor *et al.* (2003) recorded a maximum diameter of 74 mm at 150 kg N ha^{-1}. Soil amendment with black soldier fly and chitin-rich pupae exuviae significantly influenced bulb diameter and weight compared to results from previous studies. The significant increase in mean bulb diameter and weight values at each chitin level can be attributed to increased N abundance in the soil. Previous studies have reported a significant increase in mean onion bulb weight and diameter with an increase in soil N levels (Bezabih & Girmay, 2020; Piri & Naserin, 2020).

The study reported a significant increase in the onion yield due to BSF frass fertilizer and pupae exuviae. Amending the soil with black soldier fly frass fertilizer and 8% chitin increased the total biomass and shoot biomass by 46.9% and 35%, respectively. This could be attributed to high N levels of 2.91% in BSF frass fertilizer and 6.55% in pupae exuviae. The results are comparable with previous studies where 138 kg N ha^{-1} resulted in a 21% increase in total biomass (Abdissa *et al.*, 2011). Previous studies have also reported a significant increase in onion plant biomass by 12% with an increase in nitrogen level from 100 to 200 kg N ha^{-1} (Rizk *et al.*, 2012). Indeed, N is an essential element of plant growth that is responsible for protein and protoplasm buildup, inducing cell division and meristematic activity, which results in an increase in plant biomass.

Amending the soil with black soldier fly and pupae exuviae had a significant effect on the total and marketable yield of onion. Plots with 4.12 t ha^{-1} black soldier fly frass fertilizer and 8% chitin recorded 51.1 t ha^{-1} total yield and 50.6 t ha^{-1} marketable yields, which was the highest increase by 50.5% and 49.6%, respectively. These results agree with previous findings where an increase in N rates from 0 to 104 kg N ha^{-1} increased the total and marketable yield of the onion by 59.0% and 53.1%, respectively (Gateri *et al.*, 2018). Abdissa *et al.* (2011) reported total and marketable yields of 37.0 t ha^{-1} and 28.0 t ha^{-1} at 138 kg N ha^{-1}, whereas Rizk *et al.* (2012) reported 13.2 t ha^{-1} and 12.5 t ha^{-1} at 150 kg ha^{-1}. BSF frass fertilizer and chitin-rich pupae exuviae have previously demonstrated a significant increase in maize yield by 7-27% (Beesigamukama *et al.*, 2020). These results can be attributed to high N, P, Cu, S, and K levels supplied by the frass fertilizer and pupae exuviae (Table 5.4).

Nevertheless, soil amendments with BSF frass fertilizer and pupae exuviae are also associated with enhanced activity of beneficial microbes in the soil, protection against soil-borne diseases and pests, thus promoting faster plant growth and high yield (Barragán-Fonseca *et al.*, 2022). Amending the soil with chitin may also act as a bio-stimulant resulting in high nutrient uptake and increased phytohormone production that induce root growth leading to high yield (Shahrajabian *et al.*, 2021). The current study demonstrated that BSF chitin-rich pupae exuviae are a vital candidate for high crop production, increased tolerance to abiotic stress and expression of defensive plant genes.

5.6 Conclusion

The current study demonstrates that amending the soil with black soldier fly frass fertilizer and pupae exuviae can increase the marketable yield of onions by 49.6%. The different amendments rates of BSF frass fertilizer and pupae exuviae showed considerable plant height, number of leaves, mean bulb weight and diameter, total yield and marketable yield. The highest yield was obtained by amending the soil with 120 kg N ha^{-1} BSF frass fertilizer with 8% chitin rates; this can be attributed to the abundance of nutrients, enhanced soil microbial activity and crop health. The high yield of onion crops indicates that BSF frass fertilizer and pupae exuviae amendments can effectively substitute commercial fertilizer with additional benefits such as environmental friendliness, affordability, enhanced tolerance against abiotic stress and promotion of crop health. The amendment can also be employed in the controlled release of nutrients to enhance nitrogen use efficiency, reduce the frequency of nitrogen application, and reduce the rate of nutrient loss to the environment. Nonetheless, further studies should be conducted to assess the impact of BSF frass fertilizer and chitin-rich pupae exuviae on soil microbes and how this interaction mitigates crop growth and yield.

CHAPTER SIX
GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

6.1 Discussion

Root herbivory by soil-borne pests causes substantial damage to crops resulting in significant yield loss. The current study recorded numerous intractable soil-borne pests foraging on onion bulbs and cabbage roots. Indeed, the detection and monitoring of soil-borne insect pests have been a critical challenge to farmers due to the negative binomial clumped distributions of these pests as well as their cryptic feeding habits (Hill, 2008). Therefore, crop infestation by soil-borne pests usually goes unnoticed until severe damage has been achieved (Nyamwasa *et al.*, 2018). Consequently, farmers tend to apply excessive doses of broad-spectrum pesticides, culminating in pesticide resistance, pest resurgences and chemical residues in food crops. Soil-borne pests also tend to have a broad host range with the ability to survive on natural plants host when favourable hosts are out of season, while others are able to undergo resting stages in the absence of favourable conditions (Nyamwasa *et al.*, 2018).

The study recorded Malbec F1, Red Creole, Mang'ola, Red Coach and Russet F1 as the most preferred onion varieties, whereas Gloria F1, Pructor F1 and Victoria F1 were the most common cabbage varieties. This could be attributed to high yield, long shelf life, early maturity and disease tolerance (Wambani *et al.*, 2004; Arim *et al.*, 2019). The majority of the farmers were male, aged between 45-54 years, whereas women and youths aged between 18 – 34 had the least participation; this could be due to high socio-economic barriers against women and youths and unrealised opportunities (Afande *et al.*, 2015; Anderson *et al.*, 2021). The majority of the farmers also owned less than one acre of land, essentially due to the expanding population, which limited the scale of vegetable production. Ownership of land has a direct positive impact on the scale of production and household income (Rao & Qaim, 2011).

The current study reports a significantly high occurrence of onion fly (*Atherigona orientalis*) and cabbage root fly/bean seed fly (*Delia platura*). The high damage score of the onion fly could be attributed to its ability to infest the bulb, which is the harvestable part of the crop. Although *Atherigona orientalis* has been reported as a significant pest of capsicum in Nigeria, the pest is only known as a minor pest of onion in the US (Ogbalu *et al.*, 2005; Suh & Kwon, 2016). Therefore, this is the first report of *A. orientalis* as a critical pest of onion in Kenya. Other soil-borne pests such as white grubs, wireworms and sap beetles are potential pests that can cause significant crop loss under favourable environments. Nonetheless, white grubs and wireworms are considered major pests of other horticultural and forest plants, causing substantial damage under heavy infestation (Andrews *et al.*, 2008; Held & Ray, 2009; Willis *et al.*, 2010; Abney & Kennedy, 2011; Eckman, 2015; Vernon *et al.*, 2016; Vernon & van Herk, 2022). The *Urophorus humeralis* had a significant damage score, especially at physiological maturity of onion, due to its association with bacterial and fungal diseases suggesting the possibility of symbiotic associations between them (Bartelt & Wicklow, 1999; Konam & Guest, 2004). The *Urophorus humeralis* also occurred in association with *A. orientalis* resulting in severe crop damage. In order to overcome yield loss due to soil-borne insects, over 90% of farmers have turned to excessive application of synthetic pesticides. Most insecticides used were pyrethroids (lambda-cyhalothrin, alpha-cypermethrin, and beta-cyfluthrin), organophosphates and neonicotinoids. Similar results have been reported in previous studies (Kilalo *et al.*, 2009; Macharia *et al.*, 2013; Nguetti *et al.*, 2018; Omwenga *et al.*, 2021), highlighting the need for sustainable pest management approaches.

The current study revealed that BSF liquid fertilizer has the potential to cause 65% and 31.2% mortality of *A. orientalis* egg and larvae. This is comparable to stimulo-deterrent diversion/push-pull strategies which reduced oviposition on onion seedlings by 96 and 58% (Cowles & Miller, 1992). In previous studies, the use of predator *Bembidion quadrimaculatum* has also been associated with a reduction in onion fly egg survival by 17-70% (Grafius & Warner, 1989). The high egg and larval mortality could be due to the abundance of chitinolytic microorganisms such as the lactic acid bacteria, Bacillus, *Pseudomonas* spp., *Flavobacterium* spp. and *Saccharomyces* spp. that can degrade eggs shells and the tender larvae cuticles (Kielak *et al.*, 2013; Swiontek Brzezinska *et al.*, 2014; Zargar *et al.*, 2015; Chen & Peng, 2019). The recorded egg and larval mortality could also be due to the direct toxicity of chitin against insects. The BSF pupae exuviae comprises about 10.2-11.9% chitin—a linear polymer of β(1-4) linked N-acetyl-D-glucosamine and D-glucosamine which yields chitosan, a purer compound with high insecticidal properties (Dutta *et al.*, 2004; Lagat *et al.*, 2021; Lin *et al.*, 2021). Therefore, the amendment of BSF frass fertilizer and chitin-rich pupae exuviae is a novel approach that can be leveraged in managing soil-dwelling pests. However, the approach could be more productive under integrated pest management.

The study also established that amending the soil with BSF frass fertilizer and pupae exuviae can have a substantial positive effect on onion yield. For instance, the fortified BSF frass fertilizer increased the total and marketable yields by 23.8 – 50.5% and 24.0 – 49.6% compared to 34.6% and 32.9%, respectively, by commercial fertilizers. Fields amended with 8% chitin levels had the highest total and marketable onion yields of 51.10 t ha^{-1} and 50.60 t ha^{-1}, which is comparable to 66.16 t ha^{-1}, 56.26 t ha^{-1}, 53.31 t ha^{-1} and 51.64 t ha^{-1}, by Korea, the US, Spain and the Netherlands (Miassi *et al.*, 2018). Therefore, BSF frass fertilizer and pupae exuviae amendments have the potential to prevent onion fly infestation and enhance yield to match the global scales of leading onion producers. This could be due to high rates of nutrients such as nitrogen, which are moderately released to the soil, with limited loss to leaching (Ristimäki & Papadopoulos, 2000; Mogren *et al.*, 2008; Ransom *et al.*, 2020). The amendment is also associated with enhanced activity of essential microbes in the soil, which are beneficial to crop growth and health (Barragán-Fonseca *et al.*, 2022; Wantulla *et al.*, 2022).

6.2 Conclusion

The survey revealed a disproportionate participation of male farmers aged 45-54 years compared to females and youths and a high preference for hybrid onion and cabbage varieties. Results from the field survey give the first report of *Antherigona orientalis* as a significant pest of onions and *Delia platura* as a potential pest of cabbage in Kenya. Other soil-borne pests identified across different agroecological zones, especially upper and lower highlands, include white grubs, wireworms, and sap beetles, with incidences ranging from 6.7–11.2%. Over 95% of farmers in the survey region rely on broad-spectrum pesticides to control soil-dwelling pests, which pose significant risks to human health and biodiversity. The study demonstrates that BSF liquid fertilizer amended with 20-100% pupae exuviae has the potential to manage onion fly with 65.0% and 31.2% egg and larval mortality. The BSF liquid fertilizer should be applied as soil drench early in the season to offer nutrients and protection against the first generation of onion fly. Nevertheless, amending the soil with 120 kg N ha^{-1} BSF frass fertilizer and 1-8% pupae chitin can increase the marketable yield of the onion by 24.0 – 49.6%, compared to 32.9% by commercial fertilizers.

6.3 Recommendations

i. Education of farmers about the impact of frequent use of insecticides on insect resistance and associated health and environmental hazards.
ii. The study recommends using BSF liquid fertilizer with at least 20% pupae exuviae quantity in managing onion flies. The liquid fertilizer should be applied as a soil drench to target eggs deposited at the base of the stem and the emerging first instar larvae.
iii. The study also recommends using BSF frass fertilizer and pupae exuviae amendments to improve the yield of onions to the standards of the highest producers globally.
iv. The study recommends rigorous classification of the prevailing soil-dwelling species of other food crops.
v. The study recommends further work to evaluate the effect of BSF frass fertilizer and pupae exuviae amendments on the soil microbial community.
vi. The study recommends elemental analysis of the soil before and after the field experiments to determine the difference in soil-nutrient profile.

vii. Further studies should evaluate the proximate and nutritional analysis of yields due to soil amendments with BSF frass fertilizer and pupae exuviae

viii. Future studies should determine the cost-benefit analysis of fortified BSF frass fertilizer in the context of pest management and crop growth/yield promotion.

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APPENDICES

Appendix 1: Bulb onion production in major counties from the year 2019 to 2020

County	2019			2020			% Of Total Value
	Area (Ha)	Volume (MT)	Value (KES)	Area (Ha)	Volume (MT)	Value (KES)	
Kajiado	669	20,645	902,250,000	992	26,580	1,026,400,000	**21.1**
Meru	587	14,612	848,105,000	740	17,368	658,880,000	**13.5**
Bungoma	832	13,610	470,875,015	675	13,814	538,315,500	**11.1**
Homabay	512	1,790	66,371,517	367	5,988	530,094,000	**10.9**
Narok	825	12,795	381,000,000	753	6,109	288,839,750	**5.9**
Mandera	-	-	-	328	4,315	190,135,000	**3.9**
Laikipia	233	3,566	138,740,000	213	3,421	168,915,000	**3.5**
Nyeri	912	11,447	353,895,000	460	5,400	162,000,000	**3.3**
Taita Taveta	230	2,950	88,500,000	302	5,340	161,900,000	**3.3**
Machakos	125	961	42,424,000	322	2,754	132,050,000	**2.7**
Kisii	172	1,980	100,703,080	131	1,269	103,288,800	**2.1**
Isiolo	267	2,792	111,680,000	189	2,358	85,320,000	**1.8**
Tana River	34	546	24,000,000	87	1,410	84,600,000	**1.7**
West Pokot	93	1,395	41,850,000	152	3,050	71,750,000	**1.5**
Siaya	202	1,035	58,320,000	101	1,281	57,790,000	**1.2**
Kitui	131	3,120	124,500,000	135	1,920	57,600,000	**1.2**
Nakuru	49	255	14,100,000	189	2,358	85,320,000	**1.8**
Kisumu	64	512	30,340,000	77	615	45,240,000	**0.9**
Nandi	40	636	27,588,500	52	1,132	39,783,250	**0.8**
Bomet	76	576	20,328,000	78	612	34,040,000	**0.7**
Murang'a	53	684	38,520,000	49	655	32,501,306	**0.7**
Kiambu	186	1,000	38,130,000	69	869	29,480,000	**0.6**
Nyandarua	55	625	24,345,000	45	600	18,000,000	**0.4**
Garissa	65	419	24,100,000	51	183	9,130,000	**0.2**
Others	45	974	53,821,862	435	5,713	260,084,510	**5.3**
Total	**6,456**	**98,924**	**4,024,486,974**	**6,992**	**115,113**	**4,871,457,116**	**100.0**

(Horticultural Crops Directorate, 2020)

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Appendix II: The questionnaire used in recording the demographic Sand pest surveillance data

Introduction

Dear respondent, my name is Lawrence Ouma, a student from the University of Nairobi, doing research on "Management of underground insect pests of cabbages using organic soil amendments". The information being collected is for research purposes only. The confidentiality of the respondents will be ensured. Your participation is entirely voluntary. Thank you for your cooperation.

Section A: Personal details

1. County of residence

2. Gender

1	Male	
2	Female	

3. Age of the farmer

1	18-24 years	
2	25-34 years	
3	35-44 years	
4	45-54 years	
5	55-64 years	
6	65 years and over	

4. Level of Education

1	None	
2	Primary School Education	
3	Secondary School Education	
4	Tertiary Education	

Section B: Agro-Climatic data

5. Agro-ecological Zones (AEZ)

1	UH1		6	LH2	
2	UH2		7	LH3	
3	UH3		8	LH4	
4	UH4		9	UM1	
5	LH1		10	UM2	

6. Altitude range

1	0-800 m	
2	801-1200 m	
3	1201-1600 m	
4	1601-2000 m	
5	2001-2400 m	
6	Above 2400 m	

Section C: Farming Practices

1. Host crop grown

1	Cabbage (*B. oleracea* var. *capitata)*	
2	Onion (*Allium cepa*)	

2. Land size dedicated to the production of the crop (s) above

1	Less than 1 acre	
2	2-4 acres	
3	5-7 acres	
4	8 acres and above	

3. Variety

4. Growth Stage

1	Seedling stage	
2	Vegetative stage	
3	Physiological maturity	
4	Commercial maturity	

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5. Number of harvests per year

1	One harvest	
2	Two harvests	
3	Three harvests	
4	Four harvests	
5	Five harvests	
6	More than five harvests	

6. Average Yield Per Season

1	Less than 1000 Kg ha^{-1}	
2	1001-10000 Kg ha^{-1}	
3	10001-20000 Kg ha^{-1}	
4	20001-30000 Kg ha^{-1}	
5	30001-40000 Kg ha^{-1}	
6	40001-50000 Kg ha^{-1}	
7	Above 50000 Kg ha^{-1}	

7. Cropping System

1	Sole Crop	
2	Mixed cropping	
3	Others (specify)	

8. Major challenges faced.

1	Insect pests and diseases	
2	Unavailability of quality seeds and high seed costs	
3	High cost of fertilizers and pesticides	
4	Cultivation is labour intensive	
5	Poorly established value chain	
6	Lack of technical knowledge on how to prepare and apply botanicals	
7	Limited supportive policies	
8	Others (specify)	

9. Awareness of below-ground pests

1	Aware	
2	Not Aware	

10. Below ground pests

1	Cabbage root fly (*Delia* spp.)	
2	Cabbage cutworm (*Agrotis* spp.)	
3	Others (specify)	

11. Pest Management

1	None	
2	Rotating with non-host crops	
3	Rogueing of infected plants	
4	Elimination of infected crop residues	
5	Destruction of relative weed hosts	
6	Traditional (such as wood ash)	
7	Botanicals	
8	Chemicals	
9	Others (specify)	

12. Chemical Pesticides used

1	Chlorantraniprole 200g/L (CORAGEN 20 SC®)	
2	Beta Cyfluthrin (ACTARA 25WG)	
3	Malathion (FEDOTHION 50 EC®)	
4	Abamectin (Dynamec 1.8 EC)	
5	Methoxyfenoxide (RUNNER 240 SC)	
6	Imidacloprid (MURCLOPRID 25 WP®)	
7	Trichlorfon (DIPTEREX 95 SP®)	
8	Lambdacyhalothrin (KARATE 2.5WG®)	
9	Others (specify)	

13. Frequency of Application

1	Weekly	
2	After every two weeks	
3	Monthly	
4	Twice per season	
5	Once per season	
6	Others (specify)	

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Section C: Insect Pest Assessment

14. Incidence

(*Tick where appropriate [✔]*)

observations	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Present [✔]																														

observations	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	Total No. of affected plants	Incidence (%)
Present [✔]																						

15. Severity

Pest damage severity score

Class Code	1	2	3	4	5
Pest damage Severity Class	No damage and infestation	Light damage and infestation <5% of plant parts damaged or infested by pest	Average damage and infestation >5 and <50% plant parts damaged	Considerable damage and infestation >50% of plants parts damaged and severe stunting or wilting	Plants with very high infestation levels and severity of damage or wilted and dead plants

Observations	1	2	3	4	5	6	7	8	9	10	Average Severity
Severity score											