PHYTOCHEMICAL CHARACTERIZATION AND BIOPESTICIDAL ACTIVITY OF AQUEOUS PLANT EXTRACTS AGAINST FRENCH BEAN THRIPS AND APHIDS

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DECLARATION

This thesis is my original work, and it has not been submitted for the award of a degree in any other university.

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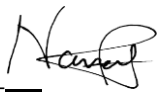
Signature: 

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DEDICATION

To my beloved parents, Mr. Robert M. Kioko and Mrs. Margaret S. Munyoki, My daughter, Binta Imani, my siblings, Diana Itumbi, Bernard Kioko, Clara Ndungwa, Brian Ngae and Christine Mueni. I appreciate the unconditional love, moral and emotional support you gave me during my studies. This made it easier for me to tackle the challenges I encountered along the way. Above all, I thank the almighty God for his protection, provision, and guidance throughout my studies.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BCMNV	Bean Common Mosaic Necrosis Virus
BCMV	Bean Common Mosaic Virus
CABI	Centre for Agriculture and Bioscience International
CMV	Cucumber Mosaic Virus
CRD	Complete randomized design
EU	European Union
FAO	Food and Agriculture Organization
FPE	Fermented plant extract
FWT	Fresh weight
H_2SO_4	Sulfuric acid
HCL	Hydrochloric acid
ICIPE	International Centre of Insect Physiology and Ecology
KALRO	Kenya Agricultural and Livestock Research Organization
LC-MS	Liquid Chromatography -Mass Spectrometry
LSD	Least significant difference
MRLs	Maximum residue limit
OACK	Organic Agriculture Centre of Kenya
RCBD	Randomized complete block design
UK	United Kingdom
UM2	Agroecological zone -Upper Midlands 2
UM3	Agroecological zone -Upper Midlands 3
UPE	Unfermented plant extract
UPE+N	Unfermented plant extract combined neem powder

ABSTRACT

In Kenya, French bean (*Phaseolus vulgaris* L.) is grown by small scale farmers mainly for exports markets. Production is majorly affected by due to moisture stress content, low soil fertility, insect pests such as bean flower thrips and black bean aphids. The use of synthetic pesticides in the management of agricultural pests over the years has been associated with many challenges such as health risks to humans and the environment. This has led to a growing interest on development of alternative strategies of plant pests' management that possesses less, to no harmful effects to the ecosystems and human health. Some of these alternative strategies include the use of plant based biopesticides such as which can be either commercial or homemade plant extracts. The plant based biopesticides have an advantage over synthetic pesticides, as they are biodegradable, and have minimal harmful effects to beneficial insects and are safer to the environment. Plants such as such as *Azadirachta indica*, *Capsicum frutescens*, *Allium sativum*, *Lantana camara* and *Tagetes minuta* have been used previously in the management of various pest by farmers individually and in mixture forms. However, information from the literature, on the efficacy of the mixture from the five plants above on aphids and thrips affecting French beans is lacking. This gap in knowledge necessitated the need to validate the efficacy of those plant extracts that organic farmers based in Kangari in Murang'a county were using on pests affecting their Frech beans, garden peas and other vegetables like cabbage in other study areas.

This study evaluated the efficacy of homemade plant extracts comprising of *Capsicum frutescens*, *Allium sativum*, *Lantana camara Tagetes minuta* and *Azadirachta indica*. The plant materials were sourced from different locations as follow; leaves of *Lantana camara* leaves, *Tagetes minuta*, were collected from ICIPE, fruits of *Capsicum frutescens* and bulbs of *Allium sativum* were purchased from local grocery stores and neem was purchased from the OACK in Kangari, Murang'a. During the efficacy trials, the plant extracts were used individually and in a mixture form where one mixture of plant extracts was fermented while the other two were unfermented. The efficacy of the above plants above were tested against bean flower thrip- *Megalurothrips sjostedti*is (Trybom) and black bean aphid - *Aphis fabae*. The efficacy of homemade plant extracts was carried out against a commercially available plant based biopesticides namely Pyeneem® (Pyrethrin 10g/L + Azadirachtin 10g/L) as a positive control. The efficacy trials were done in controlled, semi controlled, and open field environments.

The results from efficacy trials revealed that there was significant difference among various concentrations across different treatments against bean flower thrips and bean black aphid. In the

laboratory, the mean percent mortality induced by unfermented plant extract from *Tagetes minuta, Lantana camara, Capsicum frutescens, Allium sativum* combined with *Azadirachta indica* (UPE + N) against thrips was 77.3% which was comparable to that of the positive control Pyeneem® at 87.5 % A similar trends was observed in the screenhouse, In open field trials, at Chuka and Kalro Kandara, the effect of the unfermented plant extract from *Tagetes minuta, Lantana camara, Capsicum frutescens, Allium sativum* combined with *Azadirachta indica* on the mean number of bean flower thrips and that of black bean aphid was comparable to that of Pyeneem at 3.2 and 4.9 (bean flower thrips) and at 14.4 and 20.3 (black bean aphid) respectively.

The phytochemical screening of individual and mixed plant extracts was carried out using qualitative methods. However, profiling of bioactive compounds in the plant extracts associated with insecticidal activities was done using liquid chromatography mass spectrometry. Polyphenols such as flavonoids, phenolic acids, and terpenes were detected in the mixture in varies masses. Moreover, mixing the various plant extracts such as *T. minuta, L. camara, C. frutescens, A. sativum* combined with *A. indica* in tap water had a multiplicative rather than additive synergistic of the bioactive compounds as compared to the use of single plant species.

The study showed the potential of using mixed plant extracts from different plant species in the management of French beans pests. Thus, the use of plant extracts can be a useful tool in the management of pests as they can drive sustainable agriculture by promoting productivity, while preserving biodiversity which is in line with Sustainable Development Goals.

CHAPTER ONE: INTRODUCTION

1.1 Background of the study

French bean (*Phaseolus vulgaris* L.) production in Kenya is mainly done for export specifically to the EU market, with Britain being the central market (Kariuki *et al.,* 2012). French beans account for about 60 % of all vegetables exported from Kenya and small-scale farmers account for over 80 % of French bean production. Despite the good and promising market, French bean production faces many challenges, including insect pests such as bean flower thrips *Megalurobean flower thrips sjostedti* (Trybom) and black bean aphid: *Aphis fabae* (Scopoli) (Nyasani *et al.,* 2012; Fulano *et al.,* 2021). These pests attack the crop during all growing stages, leading to reduced yields and low-quality produce (Gupta and Singh, 2021).

The yield losses associated with bean flower thrips result from direct feeding, where they target leaves, flowers, and pods. Their feeding causes flower abortion, malformed pods, and leaves with white specs on the legumes, causing about 21 - 83 % losses *(Niassy et al., 2019; Reitz et al.,* 2020). Bean flower thrips have not been reported to transmit viruses, but they can spread phytopathogenic fungi and bacteria (Moritz *et al.,* 2013). The economic damage of the black bean aphid (*Aphis fabae*) is due to direct sucking, of phloem products from the leaves, soft stems of the plant, and pods, thus causing curling, yellowing, and wilting. They also cause indirect losses by the transmission of the viruses, such as bean common mosaic virus (Yadav *et al.,* 2021).

1.2 Statement of the problem

French bean production faces many challenges despite the good and promising market. The challenges include pests and diseases (Fulano *et al.,* 2021). The infestation of the crop by the pests can lead to reduced yield and low-quality produce (Gupta and Singh, 2021). Bean flower thrips can cause French bean yield losses of about 21 - 83 % if left uncontrolled (Niassy *et al.,* 2019). Black bean aphid has been reported to cause a yield losses of over 50 % on Faba beans (Skovgård and Stoddard, 2023). Most farmers use synthetic pesticides in the management of the pests affecting these pests.

The misuse and overreliance of synthetic pesticides have been associated with adverse effects to non-target organisms such as natural enemies of pests (Omwenga *et al.,* 2021), accumulation of pesticide residues in the crops biomass, soil. These pesticides residual have been reported

contaminate water bodies through water runoff, thus affecting the food chains, including humans, where they result in diseases like cancer (Lengai *et al.*, 2020; Dolma *et al.*, 2021).

Thus, there is a need for environmentally sound insect pest control strategies such as plant based biopesticides. Biopesticides can drive sustainable agriculture, which is socially acceptable by preserving biodiversity, while promoting productivity of agricultural crops, hence contributing to economic growth.

1.3 Justification of the study

The indiscriminate application of synthetic pesticides to control pests has led to residue accumulation in French bean produce, which has profound health implications. The challenges associated with the accumulation of pesticide residues in French bean produce are a significant concern, in the markets , especially in European Union and United Kingdom markets (Fulano *et al.*, 2021) . As a result, Plant protection divisions/agencies both at national and international levels have been put in place phytosanitary standards such as maximum residue levels, to monitor the quality of produce in the markets. If the MRLs are beyond the acceptable levels the produce is rejected. This has led to economic losses for farmers and the country. The accumulation of pesticide residues in the crop tissues can cause bioaccumulation of these compounds causing health hazards to humans and other organisms in the food webs.

The challenges associated with the use synthetic pesticides have given rise to the development of the alternative pests control strategies. The use of plant based biopesticides as an alternative pest control strategy especially by small-scale farmers are on the rise. However, despite the potential of plant based biopesticides to manage pests, there are drawbacks associated with their use. There are serious challenges associated with their efficacy, preparation methods, and composition and amount of their bioactive compounds of plant extracts, this informed the study.

1.4 Objectives of the study

The main objective of the study was to evaluate the potential of the mixed homemade plant extracts in the management of bean flower thrips and black bean aphid for sustainable production of French beans.

The specific objectives were:

i. To evaluate the efficacy of selected homemade plant extracts against bean flower thrips and black bean aphid in French beans.
ii. To determine the phytochemical profiles present, in the selected plant extracts

1.5 Hypotheses

i. The selected homemade plant extracts had no effect against bean flower thrips and black bean aphid in French beans.
ii. The selected plant extracts showed no phytochemical profiles.

CHAPTER TWO: LITERATURE REVIEW

2.1 French beans and their economic importance in Kenya

The optimal conditions for growing French beans (*Phaseolus vulgaris* L.) are well-drained loam soils, rainfall ranging between 900 to 1,200 mm and warm temperature ,with optimal temperature ranging from 20 to 25 ^{o} C although French beans can grow in temperatures ranging from 14 to 32 ^{o} C (Njenga, 2019). These conditions are found in high-altitude zones between 1,000 and 2,100 meters above sea level such as central, in Kiambu, Murang'a, Nyeri counties, Kirinyanga, Tharaka Nithi, parts of rift valley and western Kenya. The most common French bean varieties grown in Kenya are Serengeti, Samantha Teresa, and Paulista. They are mostly farmed for fresh bean consumption, as well as canning and freezing (Nderitu *et al.*, 2007; Njenga, 2019). French beans account for nearly 60 % of all vegetables exported from the country, primarily to the European Union market, the United Kingdom, and other countries such as China and the United States (Kariuki *et al.*, 2012; Fulano *et al.*, 2021). French bean production has numerous problems including pests and diseases despite a ready market. Insect pests such as bean flower thrips, aphids, and diseases are a major limiting factor in the production and marketing of French beans (Nyasani *et al.*, 2012).The bean flower thrips and black bean aphid infest crops at different development phases, including vegetative, flowering, and podding, resulting in reduced yield quality and quantity if left uncontrolled (Gupta and Singh, 2021).

2.2 Taxonomy, description, and distribution of bean flower thrip

Thrips belong to the order Thysanoptera; they have two suborders Terebrantia and Tubilifera which are significant agricultural pests. The bean flower thrips belong to megalurothrips genus which is further divided into *Megalutothrips usitatus* and *Megalutothrips sjostedti.* These species of thrips cause significant yield and quality losses in agricultural products worldwide (Mound and Ng, 2009). The adult female *M. sjostedti,* (bean flower thrips) measures from 1 to 2 mm while the male adult measures about 1 mm. The body color ranges from light brown to dark brown. Females can reproduce sexually or asexually, while males are haplosexual. Identification of thrips can be made using the Lucid key, which is a computer taxonomic tool as described by Moritz *et al.*, 2004. They are polyphagous pests with over a hundred host species from the temperate zone to the tropics. *Megalutothrips sjostedti,* is widely distributed in many parts of Africa, such Nigeria, South Africa, Tanzania and Kenya (Tang *et al.*, 2023).

2.3 Host range, biology and damage caused by bean flower thrips

Bean flower thrips majorly affect members of the bean family (Fabaceae), such as French beans, cowpea, and pigeon pea causing varied damages based on the susceptibility of the plant (Diabate *et al.*, 2019; Mfuti *et al.*, 2021). The majority of Thripidae (order Thysanoptera) members, including the bean flower thrips, undergo six phases in their life cycle which include; egg, two active larval stages, an inactive, non-feeding pupal stage, and an adult stage comprising of males and females (Sani *et al.*, 2017) **.** The eggs are tiny, measuring 0.25 mm in length and 0.1 mm in width. They are usually laid on the buds and calyx of developing flowers. When eggs are first laid, they are white, but they turn pale yellow color as they mature. The eggs take 2 to 3 days to hatch into the first larval instar, which is translucent in color. It takes about three days to change into a yellow 2nd larval instar. Later the 2nd larval instar develops into an inactive, non-feeding stage, known as the pre-pupal; it is followed by a pupal stage which mostly takes place in the soils (Khan *et al.*, 2022). The inactive stages take 4 to 7 days, after which adult thrips emerge from the pupa in the soil.

The first and the second larval instars actively feed on the tissue of the host plants and are partly responsible for the damage inflicted on the plant tissue. The life cycle takes about 14 to 21 days, depending on the prevailing temperatures and humidity (Sani *et al.*, 2017). Bean flower thrips have piercing and rasping mouth parts, which cause damage to the host plant directly by feeding on the plant cells' contents reducing the photosynthetic capability of the plant leaf cells. The feeding causes metabolic disorders on the plant where flower buds and flowers abort, pods deform, whereas leaves, stems, and pods are left with silvery scars thus, the plants become weak and die off.

The active larval stages cause more damage to the plants as a result of their aggregation behavior in high numbers and active feeding in certain areas such as flowers on plants due to their poor mobility. *(Abtew et al.*, 2015). The female adults also inflict direct damage to the plant tissues through oviposition punctures on the plant tissue, damaging the integrity of the plant cells. These may become entry points of secondary pathogens such as bacteria and fungi that can cause various plant diseases. The level of infestation and damage is usually high during warm seasons when the crop is at the flowering stage. However, the bean flower thrips have not been reported to transmit plant viruses (Moritz *et al.*, 2004).

2.4 Taxonomy, description, and distribution of black bean aphid

The black bean aphid -*Aphis fabae* belongs to the order Homoptera and genus *Aphis as* described by (CABI, 2022). They have a small, soft black body with a reduced exoskeleton. The legs of black bean aphids are usually pale yellow with black stripes. At the rear end of their abdomen, there is a pair of elongated slender structures like tubes, known as cornicles. The cornicles are brownish black in color and twice the length of the tail, the length of produce secretions which are waxy for defense. Cornicles in aphids are a key feature used in their identification (Michaud, 2022) .They also have piercing and sucking mouthparts which play a role in their feeding mode. The black bean aphid originated in Europe and Asia, and they have become one of the most extensively dispersed aphid species (Chalise and Dawadi, 2019). Black bean aphids are polyphagous (Pålsson *et al.*, 2020) feeding on plants from different families such as Leguminaceae, members of the bean family, and Chenopodiaceae (Pålsson *et al.*, 2020). Black bean aphids are widely distributed in both temperate and tropical regions, their spread has been hunched by their high fecundity and intrinsic reproduction rates(Chalise *et al.*, 2019).

2.5 Host range, life cycle and damage caused by the black bean aphid

Black bean aphids feeds on a wide host range of such as beans, tulip, tomatoes, peas, crucifers, beets, cucurbits, and potato (Adabi *et al.*, 2010; Łukasik *et al.*, 2022). The short lifecycle of about two weeks and feeding behaviour which is highly polyphagous (Capinera, 2020) have contributed to the success of black bean aphid as a pest. These aphids can still survive on other crops and weeds in the absence of French beans. Depending on the population density of the host plant, the black bean aphid can exist in both wingless and winged forms (Ochieng, 2022). Black bean aphids have a complicated life cycle, and, in the tropics, the female reproduces through a process known as parthenogenesis. This is a process by which the viviparous females give birth to about 20 to over 100 wingless nymphs in their lifetime. The nymphs have four stages where they molt at each stage times before transforming to an adult stage. Under favorable conditions, the young nymphs multiply and become mature adults within 10 to 14 days and begin to reproduce (Hardie, 2017).

The black bean aphid can infest plants at all growing stages, forming colonies. They are phloem feeders, causing direct damage and requiring a large amount of sap to obtain their proteins (Kumar, 2019). While feeding, they inject toxic saliva into the host plant tissues interfering with plant metabolism. The leaves curl or get twisted, the flowers abort, affecting the normal

plant growth. The honeydew secreted by aphids covers the plant leaves, reducing the surface area for photosynthesis (CABI, 2022). Lastly, black bean aphid also causes indirect damage to the plant by acting as vectors of various viruses. These viruses include the family of potyviruses such as Bean Common Mosaic Virus (BCMV), Bean Common Mosaic Necrosis Virus (BCMNV), and the family Cucumovirus - Cucumber Mosaic Virus (CMV) in common bean (*Phaseolus vulgaris*) (Worrall *et al.,* 2015; Wamonje *et al.,* 2020).

2.6 Management of the bean flower thrips and black bean aphid

There are several strategies used in the management of French bean pests. They include both preventive and curative methods. The preventive method comprises cultural strategies/control methods such as crop rotation, resistance crop varieties, field sanitation, and habitat conservation to enhance the survival of local natural enemies (Ndakidemi *et al.,* 2021). The curative strategies are employed once the insect pests have infested the host crops. They include the use of synthetic pesticides, biological control methods such as parasitoids, predators, entomopathogenic fungi, and plant based biopesticides (Ouma *et al.,* 2014; Plata-Rueda *et al.,* 2017).

2.6.1 Cultural control for the bean flower thrips and black bean aphid

Practices such as proper field sanitation, growing certified seeds free from pests and diseases, and proper soil fertility are critical in managing French beans pest. Healthy plants have a high chance of resistance against insect pests infestations compared to weak plants (Mwanauta *et al.,* 2015). Early detection of bean flower thrips and black aphid infestations is very critical to pest management success. This can be done through visual examination of the different plant parts and using sticky traps. The floral parts and leaves are tapped to detect the presence of bean flower thrips. The blue sticky traps are used for the detection and monitoring of bean flower thrips while the yellow traps are used to detect the presence of black bean aphids (Muvea *et al.,* 2014; Mfuti *et al.,* 2021). Trap crops such as stinging nettle (*Urtica dioica*) and French marigold (*Tagetes patula*) have been reported to trap bean aphids and also bean flower thrips (Sarkar *et al.,* 2018).

2.6.2 Synthetic chemical control of bean flower thrips and black bean aphid

Synthetic insecticides have been widely used in the management of insect pests as the first line of defense worldwide for food security (Baweja *et al.,* 2020). They include organophosphate, organochloride, carbamates, and synthetic pyrethroids. These pesticides have been documented

to have adverse effects on non-target organisms such as pollinators, biological control agents such as parasitoids, and predators (Nyasani *et al.*, 2015; Omwenga *et al.*, 2021). However, frequent, and indiscriminate use of synthetic insecticides in the management of bean flower thrips and black bean aphids in French beans has become a challenge. They affect the ecosystem negatively through affecting the biodiversity of non-target organisms such as natural enemies of agricultural pests, contamination of soils and water bodies through runoffs (Lebelo *et al.*, 2021). The cryptic feeding nature of bean flower thrips has also contributed to developing resistance to most synthetic chemicals (Reitz *et al.*, 2020). Therefore, incorporation of other bean flower thrips management strategies is necessary.

2.6.3 The biological control of bean flower thrips and black bean aphid

Several biological strategies are used in the management of French bean pests. These strategies include the use of natural enemies such as parasitoids, and the use of entomopathogenic fungi. Parasitoids such as *Aphidius colemani* (Mkenda *et al.*, 2019) and *Metarhizium anisopliae* isolate ICIPE 62 (Mweke *et al.*, 2018), have shown promising results in the management of aphids. On the other hand *Metarhizium anisopliae* isolate ICIPE 69, have been reported to manage *Megalurobean flower thrips sjostedti* (Trybom) effectively *(Mfuti et al.*, 2017). The use of broad-spectrum synthetic pesticides has affected biological control agents such as natural enemies negatively (Hill *et al.*, 2017). However, the process and cost of developing and proper implementation of biological control are quite high thus need to incorporate different strategies in the management of agricultural pests.

2.6.4 Botanicals as a control measure of bean flower thrips and black bean aphid

Botanicals are derivatives from different plants with pesticidal effects against various pests such as bean flower thrips and black bean aphid (Subedi *et al.*, 2018; Ahmed *et al.*, 2019). Some known plant based biopesticides include pyrethrin, which is composed of six related insecticidal compounds (pyrethrin I, pyrethrin II, cinerin I, cinerin II, jasmolin I, and jasmolin II) that occur naturally in the pyrethrum flowers (Chen *et al.*, 2018). The active compounds in pyrethrin have neurotoxic effects on insect pests, where they interrupt cation exchange of sodium and potassium ions in the voltage-gated sodium channel proteins found in insect nerve cell membranes. This leads to paralysis and the eventual death of the insect (Yang *et al.*, 2018).

Azadirachta indica-based products such as products from the seeds contain azadirachtin as active compounds. They are responsible for the neuroendocrine, respiratory interference, and antifeedant effects on various insect pests (Mordue and Nisbet, 2000). However, *Lantana*

camara has been reported to have flavones and triterpenes which have been associated with insecticidal activities against mosquito larvae (Reddy, 2013; Nyam *et al.*, 2021). Isoflavonoid rotenone compound in *Tagetes minuta* leaves has been reported to be very potent *(Gakuubi et al.,* 2016). The strong pungent smell produced by *T. minuta* repels pests, preventing them from initial attack hence discouraging the formation of colonies.

Plant extract from *Capsicum frutescens* has been documented to have repellent effects to insects pests due to the production of 8-methyl-N-vanillyl-6-nonenamide which is a capsaicin belonging to capsaicinoids group of bioactive compounds responsible for pungent smell (Baidoo and Mochiah, 2016). The bioactive compounds of *C. frutescens* are usually found on the rind and seeds of the fruit comprises of capsaicinoids, saponins, phytosterols, phenols, flavonoids, and tannins that are responsible for larvicidal action. *Capsicum frutescens* have been documented to have larvicidal effects in the control of mosquito larvae and aphids in cabbage (Parmar and Amit Gangwal, 2011). Garlic aqueous extracts have been shown to insecticidal effects on coleoptera (Dougoud *et al.*, 2019).

CHAPTER THREE

EFFICACY OF SELECTED PLANT EXTRACTS AGAINST BLACK BEAN APHID AND BEAN FLOWER THRIPS IN FRENCH BEAN CROP

3.1 Abstract

French bean (*Phaseolus vulgaris L.*) is a key export crop as it accounts for about 60 % of all vegetables exported in Kenya. Insect pests including bean flower thrips and black bean aphids remain a major challenge in the production of the crop. Broad-spectrum synthetic insecticides have been used in the management of pests over a long period of time. The overreliance of synthetic pesticides has been linked to the detrimental effects on non-target organisms and human health. This has led to the development of alternative pest management methods, such as the use of botanicals which have minimal effects on beneficial insects and are safer for the environment. This study we evaluated the efficacy of three homemade plant extracts namely, fermented plant extract from *Tagetes minuta, Lantana camara, Capsicum frutescens* and *Allium sativum* (FPE), unfermented plant extract from *Tagetes minuta, Lantana camara, Capsicum frutescens* and *Allium sativum* (UPE) and unfermented plant extract from *Tagetes minuta, Lantana camara, Capsicum frutescens, Allium sativum* combined *Azadirachta indica* (UPE + N), against bean flower thrips and black bean aphids. The efficacy trials were carried out in controlled, semi controlled, and open field environments. In the laboratory and screenhouse efficacy trials experiments, Complete random design was used for each set up and mortality data collected every day for a week. The results revealed that the efficacy of different treatments against bean flower thrip and black bean aphids varied significantly across different concentrations in different treatments. The mean percentage mortality induced by Pyeneem® against black bean aphid was comparable with that of the unfermented plant extracts combined with neem at the higher concentrations (%) of 100, 125 and 150. However, the mean percentage mortality induced by unfermented plant extracts combined with neem was significantly higher from the mortality induced by fermented plant extracts at all concentrations levels of 50, 75, 100, 125 and 150 % in the laboratory and screenhouse trials. This could have been attributed to fermentation of plant extracts in FPE resulting to loss of potency. The study showed the potential of farmers using UPE + neem in the management of French beans pests at farm level.

3.2 Introduction

Despite the good and promising market, French bean production faces many challenges, including insect pests such as bean flower thrips and black bean aphid (Nyasani *et al.*, 2012; Fulano *et al.*, 2021). The pests attack the crop during all growing stages, leading to reduced yield and low-quality produce (Gupta and Singh, 2021). The yield losses associated with bean flower thrips result from direct feeding, where they target leaves, flowers, and pods. The feeding habits of bean flower thrips causes flower abortion, malformed pods, and leaves with white specs on the legumes, causing about 21-83 % losses (Niassy *et al.*, 2019; Reitz *et al.*, 2020). Bean flower thrips have not been reported to transmit viruses, but they can spread phytopathogenic fungi and bacteria (Moritz *et al.*, 2013).

The black bean aphid causes damage to crop by both direct and indirect feeding. Direct damage occurs through feeding where the black bean aphid sucks phloem products from the leaves, soft stems of the plant, and pods, thus causing curling, yellowing, and wilting. Black bean aphid acts as vectors of viruses such as bean common mosaic virus (BCMV) and bean common mosaic necrosis virus (BCMNV). They can cause indirect damage by transmitting bean common mosaic disease which has been reported to be a very devasting disease to common beans (Yadav *et al.*, 2021; Tang and Feng, 2022). To address the challenges associated with agricultural pests, most farmers have been using synthetic pesticides as their first line of defense to manage the pests. The overreliance and indiscriminate application of synthetic pesticides to control pests has led to residue accumulation in French bean produce, which has profound health implications. The challenges associated with the accumulation of pesticide residues in French bean produce are of a significant concern, especially in the EU and UK markets which are the main markets for the product. Plant health agencies have been put in place to set standards for minimum acceptable pesticide residues. Thus, the product is subjected to tests for MRLs and if the produce doesn't meet the standards required, they are rejected. This has led to economic losses for farmers and the country.

The challenges associated with insect pests' crop yield losses and residue accumulation of synthetic pesticides have prompted small-scale farmers to look for alternative management strategies for these pests. Some of the strategies being employed include the use plant extracts such as *Allium sativum*, *Capsicum frutescens*, *Lantana camara Tagetes minuta* and *Azadirachtin indica*. The effectiveness of these plant extracts in managing the pests is relatively low despite previous studies showing the individual plants having pesticidal effects.

Thus, the current study evaluated different preparation methods of aqueous plant extracts and potential of the mixed plants in the management of bean flower thrip and black bean aphid.

3.3 Materials and methods

3.3.1 Description of the study area

Laboratory and screenhouse studies were carried out at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi. However, the on-farm studies were carried out at KALRO Kandara farm in Murang'a and Kiereni primary school farm at Chuka in Tharaka Nithi counties in Kenya. These sites lie between Nairobi and Mt Kenya region with an elevation of 1500-2000 meters above sea level. The regions have a high potential for agricultural production due to the abundant rainfall of about 1000 - 2000 mm and fertile soils that can support a wide range of crops within two cropping seasons and livestock (Place *et al.*, 2006). Chuka is situated in the upper midland - UM2 agro-ecological zone, *(*Jaetzold *et al.,* 2006) while KALRO-Kandara is situated in the upper midlands agro - ecological - UM3, *(*Jaetzold *et al.,* 2006). The soils at Chuka are humic nitisols and those at KALRO-Kandara are rhodic nitisols (Adamtey *et al.*, 2016) as described in FAO World reference base for soil resources (IUSS Working Group WRB, 2006). The site characteristics are summarized in Table 3.1

Table 3.1: Characteristic of experiment trial sites

Site	**Chuka**	**KALRO-Kandara**
Coordinates	0° 20.864' S, 37° 38.792' E	01° 0.231' S, 37° 04.747' E
Agroecological Zone	UM 2	UM 3
Altitude	1458 m	1518 m
Rainfall pattern	1373 mm	840 mm
Temperature Range	19.2 - 20.6 °C	19.5 - 20.7 °C
Cropping Season	Long Rain	Long Rain

3.3.2 Establishment of French bean host plants in the screenhouse

French beans seeds, Serengeti variety were bought from Kenya high lands company. Plastic pots measuring 10 cm in diameter and 12 cm in height were each filled with 1 kg of red loam soil mixed with poultry manure at a ratio of 3:1 this was to ensure that French beans crop is growth is not affected by excessive use of manure. Thereafter, French bean seeds were sowed in the pots and kept on a clean bench in the screenhouse awaiting germination. The seedlings were watered and maintained in the screenhouse to ensure the continuous supply of host plants for the various laboratory and screenhouse bioassays. These plants were also used to establish

and maintain thrips and aphid colonies required during various efficacy experiments in the laboratory at icipe.

3.3.3 Collection, initiation, and establishment of insect cultures in the laboratory

Aphid and thrips live insect specimens were collected from farmers French bean fields in Murang'a and Tharaka Nithi counties for the startup and establishment of colonies. French bean leaf samples infested with aphids were collected using a sharp scalpel, and samples were kept in clean plastic containers. Thrips were collected using leaf beating method on trays as described by (Bacci *et al.*, 2008) and transferred into plastic jars containing fresh French bean pods using a camel brush. The collected aphids and thrips were packed in a cooler box and transported to the laboratory.

Aphids-infested plant materials from the field were removed from plastic containers and transferred to four weeks old French bean plants as food substrates for 24 hrs. This was done to allow the black bean aphid to move to the new substrate infested plants placed in 20 x 15 x 40 cm cages for feeding and parthenogenesis. French bean pods and flowers infested with thrips from the field were removed from jars and transferred to one-liter jars containing clean, fresh French bean pods as food substrate and then covered with lids fitted by a netting material 4760 um mesh size for feeding and oviposition. Morphological identification as described by Moritz *et al.*, 2004, was done to obtain pure stock of black bean aphid. The identified black bean aphids and bean flower thrip colonies were multiplied and maintained for experimental purposes.

The insect cultures were maintained for at least three generations before the commencement of the experiment to allow the insect to adopt to the new laboratory conditions. The food substrates were changed with fresh ones regularly after 48 hrs to minimize on competition and enhance colony buildup. Proper sanitation of the rearing units was maintained to avoid infection of the colonies by pathogens or predators. In addition, the insect cultures were periodically replenished with fresh field cultures to maintain the vigor of the stock.

3.3.4 Experimental design and layout

Complete Random Design (CRD) was used to carry out both laboratory and screenhouse experiments. The choice of the design was guided by the homogeneity of the test sample, treatment, and the environment (Sisay *et al.*, 2019). Treatments in the laboratory and

screenhouse experiments comprised of fermented plant extract from *Tagetes minuta, Lantana camara, Capsicum frutescens* and *Allium sativum* (FPE); Unfermented plant extract from *Tagetes minuta, Lantana camara, Capsicum frutescens* and *Allium sativum* (UPE), and Unfermented plant extract from *Tagetes minuta, Lantana camara, Capsicum frutescens, Allium sativum* combined *Azadirachta indica* (UPE + N). A commercial botanical called Pyeneem® that contained Pyrethrins 10g/l and Azadirachtin10g/l as the main active compound was used as positive control. In addition, a negative control consisting of composed of tap water was used in the experimental setup. In addition to the treatments tested above, the single plant extracts were evaluated for their efficacy in the laboratory. The experimental set up was replicated four times.

Randomized complete block design (RCBD) was adopted for open field experiments, to take care of variations due to environmental factors such as soil fertility during the experiment. Treatment used in the field experiment comprised of unfermented plant extract from *Tagetes minuta, Lantana camara, Capsicum frutescens, Allium sativum* combined with *Azadirachta indica* (UPE + N), a commercial botanical biopesticide called Pyeneem® used as positive control and a negative control that contained no treatment (Miller *et al.,* 2020).

These treatments were sourced from various locations as follow; Pyeneem® a commercial botanical, was sourced from Juanco ltd. Components of plant extracts such as leaves of *Lantana camara* leaves, *Tagetes minuta,* were collected from ICIPE, fruits of *Capsicum frutescens* and bulbs of *Allium sativum* were purchased from local grocery stores and neem was purchased from the OACK in Kangari, Murang'a. There was no standardization of materials used for preparation of plant extracts in terms of actively growing them in a certain agroecological zone. They were sourced from the local market and the surrounding environment just like the farmer's practice. The preparation of the different plant extracts for the efficacy trials was guided by the proportions farmers were using to prepare the fermented plant extract. This was necessary for validation purposes.

3.3.5 Preparation of fermented plant extract

Four hundred grams of fresh leaves of *Tagetes minuta* and *Lantana camara* respectively were shredded into small pieces and put into a five-liter bucket. In addition, 200 g of *Capsicum frutescens* and *Allium sativum* each were crushed into fine paste. This was done to minimize the irritation effects of the two plants on the user. The paste was mixed into the shredded pieces

of *T. minuta*, and *L. camara* leaves in a five-liter bucket. Fifty milliliters of molasses and two liters of tap water were added to the mixture which was left for 14 days to ferment. Later the mixture was sieved through a fine piece of cloth of 4760 um mesh size. Ten milliters of Teepol which is a liquid soap was added to the extract to act as an adjuvant. The fermented plants extract formulation was presumed to have a concentration of 100 % (0.60 g/ml) of the standard treatment. Thereafter, four more concentrations were prepared from (0.60 g/ml) as defined above through serial dilutions at the rate of 50 % (0.30 g/ml), 75 % (0.45 g/ml), 125 % (0.75 g/ml) and 150 % (0.90 g/ml) giving a total of five concentrations replicated four times.

3.3.6 Preparation of unfermented plant extract

The individual plant extracts making up the fermented plant extracts in 3.3.5 and additional neem extracts were prepared. Four hundred grams (0.2 g/ml) of T. *minuta and L. camara* young leaves each were cut into small pieces and put in a small bucket separately. Thereafter, 200 grams (0.1g/ml) of *C. frutescens* fruits and *A. sativum* bulbs each were crushed into a fine paste. Thereafter 100 grams (0.05 g/ml) of *A. indica* was measured and soaked in tap water. Each plant extract was soaked in two liters of tap water for 24 hrs, thereafter the solution was sieved and used for the efficacy experiments.

A second set of unfermented plant extract was prepared using the same components just like the ones used in fermented plant extract except that the molasses was not added, and the plant extract was soaked in tap water for 24 hrs instead of 14 days as in the case of fermented plant extracts in 3.3.5 above. This was carried out to compare the effect of fermentation on the efficacy of the plant extracts against bean flower thrips and black bean aphids on French beans. The plant extract was prepared as follows, 400 g of fresh leaves of *L. camara* and *T. minuta* respectively were cut into small pieces and put into a five-liter bucket. Thereafter, 200 g of *C. frutescens* and *A. sativum* each were crushed into a fine paste. The paste was mixed with the shredded pieces of *T. minuta*, and *L. camara* and two litres of tap water were added to the mixture. The resulting mixture was sieved through a fine net and one percent of Teepol® a liquid soap was added to the extract to act as an adjuvant. The unfermented plant extracts formulation was presumed to have a concentration of 100 % (0.60 g/ml) as the standard treatment. Thereafter, four more concentrations were prepared from the stock solution (0.6 g/ml) as defined above through serial dilutions at the rate of 50 % (0.3 g/ml), 75 % (0.45 g/ml), 125 % (0.75 g/ml) and 150 % (0.9 g/ml) giving a total of five concentrations replicated four times.

Another set of unfermented plant extract just like the above except that 100 grams of neem cake powder was incorporated, to the mixture. This was done to assess if neem improved the efficacy of the unfermented plant extract against the pests. The resulting mixture was sieved through a fine net and 1 % of Teepol® a liquid soap was added to the extract to act as an adjuvant. The resultant extract formulation was presumed to have a concentration of 100 % (0.65) of the standard treatment. Four more concentrations were prepared from the standard stock (0.65 g/ml) through serial dilutions at the rate of 50 % (0.33 g/ml), 75 % (0.49 g/ml), 125 % (0.81 g/ml) and 150 % (0.98 g/ml) giving a total of five concentrations replicated four times.

3.3.7 Evaluation of the efficacy of selected botanicals against bean flower thrips

In the laboratory, four pods were placed in a jar per treatment and infested with 80 adult bean flower thrips which were two days old using a fine camel brush. and allowed to settle for 24 hrs before treatment application. The infested pods with bean flower thrips were then sprayed once with five milliliters of the treatment per jar in respective concentrations using a hand sprayer. Mean percent mortality data was recorded daily after 24 hrs post-treatment for seven days.

Under screenhouse conditions, the efficacy trial was carried out as follows; Four French bean pods were placed in a jar and thereafter infested with 80 bean flower thrips adults using a fine camel brush and allowed to settle for 24 hours before treatment application. The infested pods with bean flower thrips were sprayed once with five milliliters of the treatment per jar in respective concentrations using a hand sprayer. Mortality data was recorded daily after 24 hrs post-treatment for seven days. Pyeneem® was used as positive control while tap water was used as negative control in the experimental set up.

3.3.8 Evaluation of the efficacy of selected botanicals against black bean third instar aphid nymph

Thirty healthy, eight weeks old French bean plants were used in the laboratory experiment per treatment and each plant was infested with 80 aphid nymphs using a fine camel brush. After infestation, the plants were placed inside perspex cages measuring 20 x 20 x 30 cm with a fine netting material with mesh size of 4760 um before treatment application. The infested plants with aphids were sprayed with 10 ml of respective treatment concentrations covering all the leaves on the plant using a hand sprayer. Mortality data was recorded daily after 24 hrs post-treatment for seven days.

Under the screenhouse setting, the efficacy trial of the botanicals was carried out using 30 French bean plants which were eight weeks old. Healthy, French beans plants free from pest infestation were selected and assigned to the different treatments described above randomly. A fine camel brush was used to infest each plant with 80 aphid nymphs. The plants were placed in perspex cages measuring 20 x 20 x 30 cm, with a netting material with a mesh size of 4760 um before application of the treatment. The aphid nymphs were allowed to settle on the plant for 24 hrs before applying the different treatments. About 10 ml of each respective treatment was applied to the plants using a hand sprayer. The data on mortality was recorded after 24 hrs of treatment daily for seven days. Pyeneem® was used as positive control while tap water was used as negative control.

3.3.9 Determination of the efficacy of selected botanicals against bean flower thrips and black bean aphid under open field conditions.

The best-performing homemade plant extract from laboratory and screenhouse experiments (UPE+N) was selected and tested for efficacy against bean flower thrips and black bean aphid in the open field. One positive control (Pyeneem®), and a negative control (where no treatment was applied) were considered in the experiment. The field experiment was established in the long rainy season between March and June 2022. Soil samples from identified experimental areas were collected and analyzed before randomizing treatments to ensure proper and balanced placement of treatments. Sampling was done at depths of 0-20 cm, and soil samples were analyzed for macro and micronutrients. Initial soil chemical composition results were used to calculate the required nutrient balances from the various inputs.

Land preparation was done by hand hoeing after which the experimental area was divided into four blocks in each site. Each experimental block consisted of three plots, with each plot measuring 3.5 x 3.5 meters with separation path measuring 1 m on each side, giving a total of 12 experimental plots per site. The three treatments, namely unfermented plant extract combined with neem, Pyeneem® and a control where no treatment was applied, were randomly placed in RCBD. Planting furrows were made on each plot at a spacing of 30 cm from one row to another. Inputs were placed inside the furrows starting with compost, followed by rock phosphate after dissolution with citric acid. Thereafter all the inputs were mixed with soil before seed sowing (Table 3.4 and Table 3.5). French bean seeds, two seeds per hole, were sown at a spacing of 15 cm by 30 cm. Germination count and thinning were done two weeks after germination to maintain the required plant population per plot. Topdressing with nitrogen biofertilizer was done at vegetative and reproductive crop stages using *Tithonia diversifolia* plant tea (Table 3.2. and Table 3.3). Hand weeding was also carried out regularly to minimize nutrient and soil moisture competition from weeds.

Table 3. 2: Biofertilizer management plan for Chuka site

Inputs at Planting	**Amount FWT Kg/ha**	**Amount/ plot (Kg)**
Compost (30.28N: 9.28P)	5500	6.74
Tithonia mulch -FWT (7.45 N:1.35 P Kg/ha)	1687.9	2.07
Phosphate rock (23.40 P Kg/ha)	195	0.24
Citric acid powder (Kg)	48.75	0.06
Inputs at five weeks after planting	**Amount FWT Kg/ha**	**Amount/ plot (Kg)**
Tithonia tea (37.65N: 6.83 P Kg/ha)	8535.03	10.46
Phosphate rock (20.40 P Kg/ha)	170	0.21
Citric acid powder (Kg)	42.5	0.05
Input at 50% podding stage	**Amount FWT Kg/ha**	**Amount/ plot (Kg)**
Tithonia tea (37.65N: 6.83 P Kg/ha)	8535.03	10.46
Total amount of N and P required by the crop (N113.03: 68.10 P Kg/ha)		

Table 3.3: Biofertilizer management plan for Kalro-Kandara site

Inputs at Planting	**Amount FWT Kg/ha**	**Amount/ plot (Kg)**
Compost (40.03 N: 17.85 P)	5500	6.74
Tithonia mulch -FWT(Kg/ha)	0	0
Phosphate rock (16.08 P Kg/ha)	134. 00	1.16
Citric acid powder (Kg)	33.5	0.04
Inputs at five weeks after planting	**Amount FWT Kg/ha**	**Amount/ plot (Kg)**
Tithonia tea (36.50 N: 7.10P Kg/ha)	18750	10.72
Phosphate rock (19.8 P Kg/ha)	165	0.2
Citric acid powder (Kg)	41	0.05
Input at 50% podding stage	**Amount FWT Kg/ha**	**Amount/ plot (Kg)**
Tithonia tea (36.54 N: 7.14 P Kg/ha)	8750	10.72
Total amount of N and P required by the crop (N113.03: 68.10 P Kg/ha)		

Pest scouting was done two weeks after the emergence of the French bean plants while the subsequent scouting's were carried out once every week. During scouting and sampling for bean flower thrips from the French bean plants, each experimental plot was sub-divided into four quadrants. Five plants were sampled randomly in each quadrant using beating method. The plants bean flower thrips were collected over a white tray sprayed with 70 % ethanol to prevent bean flower thrips from flying away. This was done for approximately one minute to dislodge bean flower thrips from the plant. This method permitted collection of both adults and immature stages of bean flower thrips. Bean flower thrips collected were transferred into labeled 50 ml falcon tubes containing 70 % ethanol. During flowering stage of the crop, each plot was divided into four quadrants, five plants were selected randomly and in each plant two fully opened flowers were picked for sampling giving a total of 40 flowers per plot. These flower samples were then put in labelled falcon tubes containing 70 % ethanol. All the samples were taken to the laboratory for counting and morphological identification using the LucID key (Muvea *et al.*, 2014; Nyasani *et al.*, 2015). The sample collection was carried out until the crop reached the senescence stage.

Similarly, to bean flower thrips, aphids from the French bean crop were scouted and collected. The plots were divided into four quadrants and five plants were sampled randomly in each quadrant. However, contrary to the bean flower thrips above where beating method was used, aphids were scouted and collected from two leaflets on the upper, middle, and lower parts of

the plant. Using a fine camel brush, aphids were dislodged from the host plants parts into labelled 50 ml falcon tubes containing 70 % ethanol. Thereafter, these samples were taken to the laboratory for counting and identification.

3.3.10 Data management and analysis

The generated data was analyzed using R software (version 4.2.2) R Core Team (2023). The mortality data from the laboratory and screenhouse experiments was subjected to normality test using Shapiro test (Shapiro and Wilk, 1965). The mortality data for control treatment was corrected using Abbott's formula (Abbott, 1925; Kerns *et al.*, 2022) as follows

$$\text{Abbott formula} = \frac{(\%\ \text{mortality of the test subject} - \%\ \text{control mortality}}{(100 - \text{control mortality x } 100)}$$

then arcsine transformed before being subjected to Analysis of variance (ANOVA) and means were compared and separated using the least significant difference (LSD) test at 0.05 significance level (Searle *et al.*, 1980). Negative binomial regression was used to compare the number of bean flower thrips and aphids collected from different plants sampled from Chuka and Kalro-Kandara due to over dispersion of the data (Fávero *et al.*, 2020). During the analysis, the glm. nb function in the MASS package and Anova function in the car package were used for analysis of deviance, and the nagelkerke function was used to determine p-value and pseudo-R-squared value for the model. Post-hoc analysis was conducted with the emmeans package in R environment (R Core Team 2023).

3.4 Results

3.4.1 The effect of different plant extracts against bean flower thrips

In the laboratory, all the treatments induced mean percent mortality on bean flower thrips. However, mean percent mortality induced by the unfermented plant extract combined with neem (UPE + N) was significantly different higher ($P < 0.05$) compared to the mean percent mortality induced by the individual plant extracts. The unfermented mixed plant extract combined with neem (UPE + N) induced a mean percent mortality of 64 % while *Lantana camara* induced a mean percent mortality of 42 %. All other individual plant extracts induced a mean percent mortality of less than 50 % except *Azadirachtin indica* which induced a mean percent mortality of 54 % (Figure 3.1).

On mixing various plant extracts under different preparation methods, all the treatments under different concentrations induced mean percent mortality on bean flower thrips which was significantly different ($P < 0.05$). The mean percent mortality of bean flower thrips increased with increased concentration of different treatments. The mortality induced by the unfermented plant extracts combined with neem (UPE + N) was comparable with that of positive control treatment (Pyeneem®) at concentration 75,100 and 150 %. However, the mean percent mortality induced by the same treatments at concentration 50 and 125 % were significantly different ($P < 0.05$). At 125 % Pyeneem® and UPE +N induced a mean percent mortality of 82.5 and 77.3 % respectively. In contrast, the fermented plant extract (FPE) did not induce mortality above 50 % at any concentration (Table 3.4).

In the screenhouse conditions, there were significant differences among the mean percent mortalities induced by various concentrations across the different treatments ($P < 0.05$). The mean percent mortality induced by the unfermented plant extracts combined with neem (UPE + N) was not significantly different from the mean percent mortality induced by Pyeneem® under various concentration levels except at 100 %. However, at concentration level 150 %, the mean percent mortality induced by Pyeneem® at 71.3 % and unfermented plant extract combined with neem (UPE + N) at 65 % was significantly different from the mortality induced by the fermented plant extracts (FPE) at 21.8 % which did not induce mortality above 50 % at any concentration (Table 3.5).

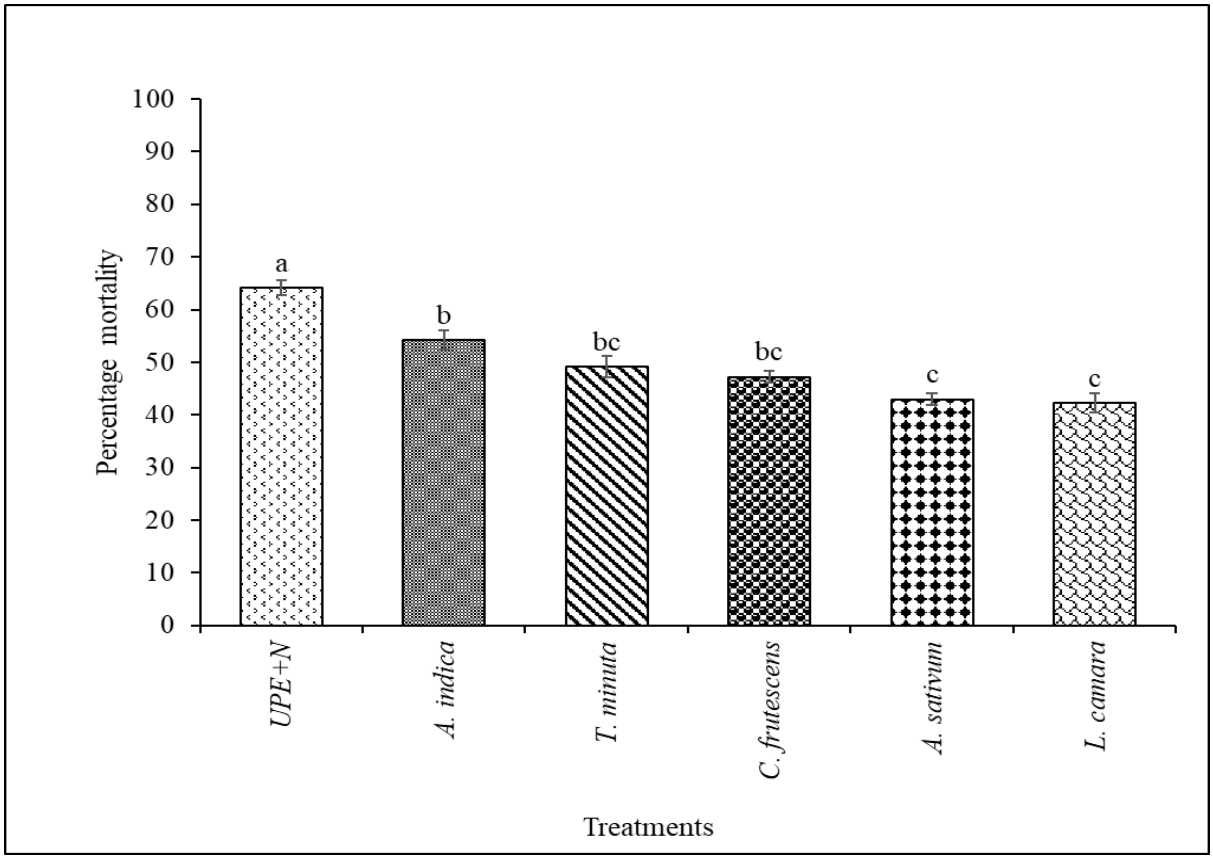

Figure 3.1: Mortality of bean flower thrips induced by individual plant extracts in the laboratory. UPE+N =Unfermented plant extract combined with neem powder

Table 3.4: Mortality of bean flower thrips induced by mixed plant extracts in the laboratory

	Concentration (100%)				
Treatment	50	75	100	125	150
Pyeneem®	27.8 ± 0.4a	47.3 ± 0.8a	65.8 ± 0.4a	82.5 ± 1.0a	87.5 ± 1.5a
UPE + N	21.5 ± 0.8b	44.8 ± 1.0a	61.5 ± 1.8a	69.3 ± 2.6b	77.3 ± 2.6a
UPE	11.8 ± 1.0c	27.0 ±0.7b	38.3 ± 1.4b	54.0 ± 1.2c	58.0 ± 2.1b
FPE	3.3 ± 0.9d	4.8 ± 1.1c	6.3 ± 1.4c	13.3 ± 2.4d	18.8 ± 0.8c
LSD	4.78	5.46	8.12	11.64	11.15
CV %	22.18	13.15	14.1	15.85	13.77

All alphabets in small letters (a, b, c) show mean separation across concentration of different treatments at ($p < 0.05$). UPE+N =Unfermented plant extract combined with neem powder, UPE= Unfermented plant extract, FPF= fermented plant extract.

Table 3.5: Mortality of bean flower thrips induced by mixed plant extracts in the screenhouse

	Concentrations (%)				
Treatment	50	75	100	125	150
Pyeneem®	17.8 ± 0.4a	40.5 ± 0.4a	50.3 ± 0.4a	65.8 ± 1.3a	71.3 ± 0.8a
UPE + N	16.0 ± 0.9a	35.8 ± 1.3a	35.8 ± 1.4b	55.5 ± 2.6a	65 ± 1.3a
UPE	10.0 ± 0.7b	23.8 ± 0.6b	32.5 ± 1.2b	51.8 ± 2.9a	48.8 ± 2.5b
FPE	4.8 ± 0.4c	7.5 ± 1.5c	11.5 ± 1.2c	23.0 ± 2.3b	21.8 ± 2.2c
LSD	3.8	6.47	6.77	14.04	10.81
CV %	23.4	17.95	15.53	21.37	15.59

All alphabets in small letters (a, b, c) show mean separation across concentration of different treatments at ($p < 0.05$). UPE+N =Unfermented plant extract combined with neem powder, UPE= Unfermented plant extract, FPF= fermented plant extract.

3.4.2 The effect of different plant extracts against black bean aphid

In the laboratory, all the treatments induced mortality of black bean aphid. However, the unfermented plant extracts combined with neem (UPE + N) induced the highest mortality at 68.8 % among all the treatments. Moreover, there were significant differences ($P < 0.05$) among the mean percentage mortalities induced by unfermented plant extracts combined with neem at 68.8 %, *A. indica* at 55 % *and L. camara* 46.5 %. The mean percent mortalities induced by *A. indica* at 55 % and *T. minuta* 54 % were not significantly different from *C. frutescens* at 49.5 % (Figure 3.2).

In the laboratory, there were significant differences ($P < 0.05$) among the mean percent mortalities induced by the various concentrations within different treatments on black bean aphids. At concentration of 150 % Pyeneem® induced the highest mean percent mortality of black bean aphid at 78 % which was not significantly different from the mean percent mortality induced by the unfermented plant extracts combined with neem (UPE + N) at 75 %. However, the fermented plant extract (FPE) did not induce a percent mortality of more than 50 % at any concentration (Table 3.6).

In the screenhouse, all treatments induced mortality of black bean aphid. The mean percent mortality induced on the black bean aphids increased with treatment concentrations. However, at all concentration levels, the mean percent mortality induced by Pyeneem®, and UPE + N on black bean aphids under screenhouse conditions was lower than the mortality induced in the laboratory see (Table 3.6 and Table 3.7).

The unfermented plant extracts combined with neem (UPE + N) at concentration level of 150 % induced the highest mean percent mortality of at 71 % although it was not significantly different to that of Pyeneem®. However, there was significant differences ($P < 0.05$) among mortalities induced by different plant extracts on black bean aphid at different concentration levels expect at concentrations 75 % where the mortalities induced by UPE +N and UPE were not significantly different (Table 3.7).

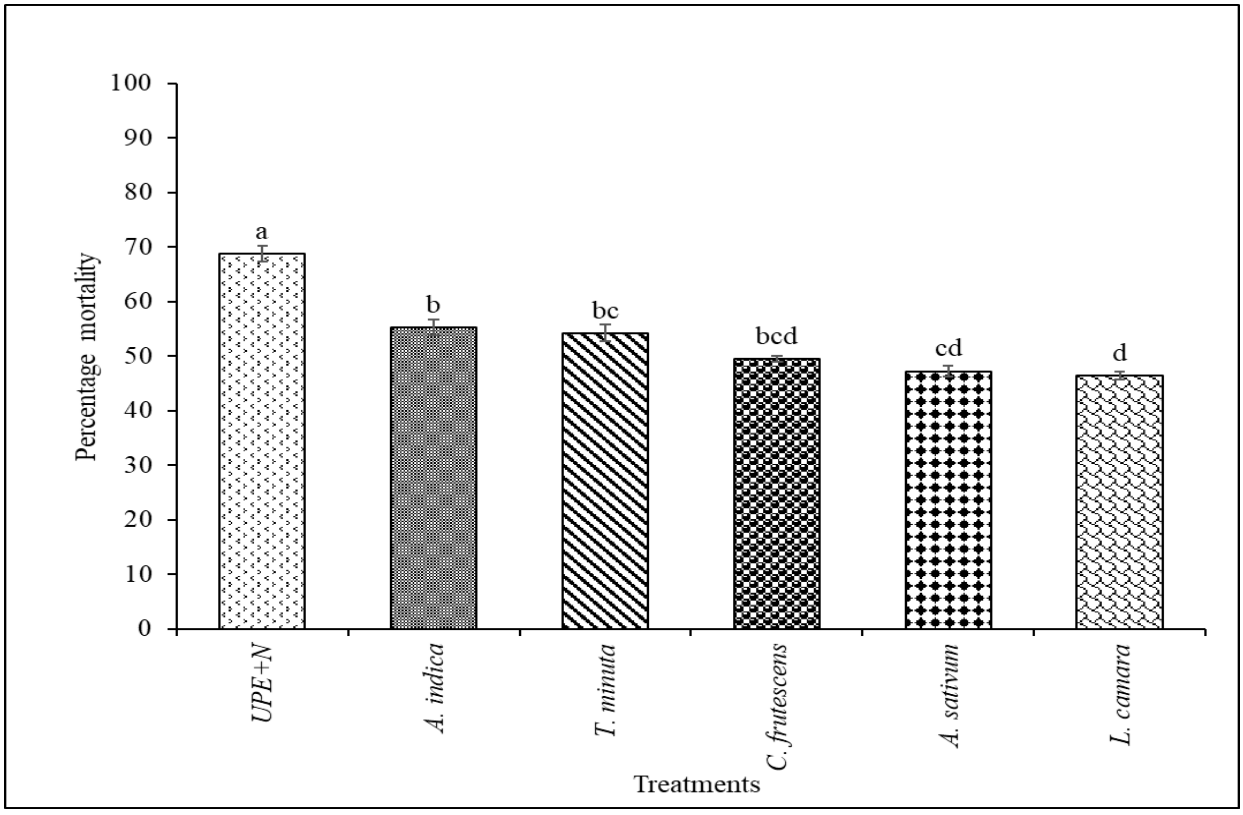

Figure 3.2: Mortality of induced by black bean aphid individual plant extracts in the laboratory. UPE+N =Unfermented plant extract combined with neem powder

Table 3.6: Mortality of black bean aphid induced by mixed plant extracts in the laboratory

	Concentration (%)				
Treatment	50	75	100	125	150
Pyeneem®	11.5 ± 0.6b	38.3 ± 0.4a	56.3 ± 1.3a	59.3 ± 1.3a	78 ± 1.5a
UPE + N	19.3 ± 0.5a	26.8 ± 0.8b	56.3 ± 1.3a	57.3 ± 1.6a	75.0 ± 0.6a
UPE	7.5 ± 1.3bc	14.0 ± 1.3c	28.5 ± 1.2b	48 ± 0.7c	59.5 ± 1.0b
FPE	4.5 ± 0.6c	10.3 ± 0.3c	12.3 ± 0.7c	21.3 ± 1.5c	22.5 ± 0.8c
LSD	5.05	4.74	7.75	7.96	6.19
CV %	35.23	15.85	15.71	12.79	7.86

All alphabets in small letters (a, b, c) show mean separation across concentration of different treatments at ($p < 0.05$). UPE+N =Unfermented plant extract combined with neem powder, UPE= Unfermented plant extract, FPF= fermented plant extract.

Table 3.7: Mortality of black bean aphid induced by mixed plant extracts in the screenhouse

	Concentration (%)				
Treatment	50	75	100	125	150
Pyeneem®	9.3 ± 0.4b	28.8 ± 0.9a	41.3 ± 0.6a	51.3 ± 1.5ab	68.0 ± 2.5a
UPE + N	12.5 ± 0.9b	14.5 ± 1.0b	32.8 ± 0.6a	56.3 ± 1.1a	71.0 ± 1.4a
UPE	21.3 ± 0.3a	15.0 ± 0.6b	13.0 ± 3.2b	43.0 ± 2.1b	44.0 ± 2.2b
FPE	3.3 ± 0.0c	7.8 ± 1.2c	11.5 ± 0.9b	20.5 ± 0.8c	24.5 ± 1.5c
LSD	3.66	5.56	10.34	8.77	11.79
CV %	23.62	25.13	31.31	15.31	16.95

All alphabets in small letters (a, b, c) show mean separation across concentration of different treatments at ($p < 0.05$). UPE+N =Unfermented plant extract combined with neem powder, UPE= Unfermented plant extract, FPF= fermented plant extract.

3.4.3 Effect of unfermented plant extract combined with neem against bean flower thrips and black bean aphid in open field at Chuka and Kalro-Kandara

There was a significant reduction of mean number of black bean aphids and bean flower thrips in plots treated with unfermented plant extracts combined with neem (UPE +N) and Pyeneem® compared to the control where no treatment was applied at Chuka and Kalro-Kandara. Generally, at Kalro-Kandara, the mean number of bean flower thrips and black bean aphids in both treated and controlled plots was higher compared to Chuka. There was significance difference in the mean number of bean flower thrips and black bean aphids ($p<0.05$) among the treated and control plots. At Kalro-Kandara the mean number of black bean aphid in plots treated with UPE + N was significantly lower than that of control plots in the same site. Similarly, the same trend was observed in the mean number of black bean aphid at Chuka. However, there was no significant difference between the UPE +N and Pyeneem® in terms of reducing the mean number of bean flower thrips and black bean aphid in both sites (Table 3.8). At Chuka the mean number of bean flower thrips in the plots treated with unfermented plant extract combined with neem (UPE + N) was lower than that of Kalro-Kandara at 3.2 and 4.9 respectively. Similarly, there were higher mean number of black bean aphids at Kalro-Kandara compared to Chuka (Table 3.8).

Table 3.8: Mean number of black bean aphids and bean flower thrips collected from different treatments at Chuka and Kalro-Kandara

Treatment	Black bean aphids		Bean flower thrips	
	Chuka	Kalro-Kandara	Chuka	Kalro-Kandara
UPE + N	14.4 ± 4.2a	20.3 ± 8.5a	3.2 ± 0.4a	4.9 ± 0.7a
Pyeneem®	21.4 ± 5.7a	31.1 ± 9.3a	4.6 ± 0.7a	3.9 ± 0.5a
Control	99.7 ± 19.2b	157.6 ± 31.2b	13.9 ± 1.9b	15.1 ± 1.6b
LR Chisq	35.379	26.142	58.059	73.779
Pr (>Chisq)	0.05	0.05	0.05	0.05

All alphabets in small letters (a, b, c) show mean separation across concentration of different treatments at ($p < 0.05$). UPE+N =Unfermented plant extract combined with neem powder, UPE= Unfermented plant extract, FPF= fermented plant extract.

3.5 Discussion

The study revealed that the mean percent mortality induced by unfermented mixed plant extracts combined with neem (UPE + N) was comparable to Pyeneem® in the laboratory, screenhouse and open field experiment. Although in some cases Pyeneem® induced higher mean percent mortality of bean flower thrips and black bean aphid compared to UPE +N. This could be attributed to the fact that Pyeneem® was more refined as a commercial product compared to UPE + N which is a homemade product. The performance of Pyeneem® and UPE +N against bean flower thrips and black bean aphid could have been attributed to their bioactive compounds. Pyeneem® is composed of Pyrethrin 10g/L + Azadirachtin 10g/L as bioactive compounds.

Pyrethrins are natural bioactive compounds from pyrethrum plants. They are widely used in the management of the insect pests (Khater, 2012; Oguh *et al.*, 2019) .They target nervous system, which may lead to paralysis resulting into ultimate death of the insect (Soderlund, 2020). Combining pyrethrins and azadirachtin as a bioactive compound could have contributed to efficacy of Pyeneem® against bean flower thrips and black bean aphid in the current study. These results concur with previous studies where the combination of pyrethrin and azadirachtin bioactive compounds was effective in the management of bean flower thrips (Iglesias *et al.*, 2021). Thus Pyeneem® could be incorporated in the management strategies for bean flower thrips and black bean aphid.

The unfermented mixed plant extracts combined with neem (UPE + N) showed promising results as compared to the use of a single plant extract in the management of bean flower thrips and black bean aphid. The UPE + N consisted of; *Lantana camara, Tagetes minuta, Azadirachta indica, Capsicum frutescens* and *Allium sativum* plant materials. These plant materials are composed of varied bioactive compounds with insecticidal activities. The active compounds in neem are azadirachtin, nimbidol nimbolinin quercetin nimbin among others. These compounds are known to be repellent, antifeedant, interfere with reproduction and feeding of insects (Liang *et al.*, 2003; Sow *et al.*, 2015; Ghoneim *et al.*, 2017) They also interfere with hormone responsible for molting in insects (Chaudhary *et al.*, 2017). On the other hand, *L. camara*, contains lantadane and triterpenoids which have antifeedant activities (Dua *et al.*, 2010). *Tagetes minuta* has been reported to have repellent effects on insects (Phoofolo, 2013). Therefore, these multiple modes of action could have had synergistic effect and could

have contributed to the observed efficacy of UPE + N against thrips and aphids on French beans even though the exact mode of action was not evaluated in this study.

The unfermented plant extract combined with neem, and Pyeneem® induced the highest percentage mortalities against both bean flower thrips and black bean aphid both in the laboratory and screenhouse. In the open field set up, the plots which were treated with the two treatments, recorded the lowest mean number of bean flower thrips and black bean aphids. The two botanicals contained *Azadirachta indica* bioactive compounds such as Azadirachtin, which have been reported to effect against aphids. other studies (Pissinati and Ventura, 2014; Nahusenay, 2020). Azadirachtin is a complex bioactive compound with insecticidal effects. Azadirachtin has a structure which resembles that of ecdysone hormone which is responsible for insect metamorphosis. This enables the bioactive compound to bind to the insect receptors such as taste receptors in the mouthparts. The signal is encoded in the central nervous system thus sending signals to stimulate the chemoreceptors especially the deterrent cells to block feeding stimulation in the insect (Chaudhary *et al.*, 2017) .

The use of different preparation methods in different plant extracts contributed to their varied efficacy against bean flower thrips and black bean aphid under laboratory and screenhouse conditions. The fermented mixed plant extracts induced mean percent mortality of below 50 % both in laboratory and the screenhouse experiments. This could have been attributed to the biodegradation of active compounds due to environmental factors such as sunlight. Therefore, there is a need to evaluate the best combination of active ingredients and the best preparation method. Even though the process of preparing the crude plant extracts is laborious and time consuming, the benefits of sustainable and environmental friendliness give them a competitive advantage. This is more so in vegetables that are harvested regularly since there are no post-harvest intervals required.

3.6 Conclusion

The unfermented plant extract combined with neem had insecticidal activities against bean flower thrips and black bean aphids. The percentage mortality induced by the unfermented plant extract combined with neem, was comparable to that induced by a commercial product, Pyeneem®. Thus, the unfermented plant extracts combined with neem, can be used in the management of bean flower thrips and black bean aphid in French bean farming. This could reduce the risks involved in the indiscriminate use of synthetic pesticides in French bean production.

CHAPTER FOUR

PHYTOCHEMICAL PROFILLING OF SELECTED AQUEOUS PLANTS EXTRACTS ASSOCIATED WITH INSTECTICIDAL ACTIVITIES

4.1 Abstract

The use of plant extracts in agricultural pests' management is gaining a lot of attention due to their potential effectiveness, minimal environmental and human health hazards. Their use has been affected by many challenges such as variable efficacy due different composition of bioactive compounds, development of resistance and target specificity. However, information on the synergistic or antagonist effects on the bioactive compounds after mixing *A. indica*, *C. frutescens*, *A. sativum*, *L. camara* and *T. minuta* in water as a solvent is lacking. This study therefore was designed to profile the various phytochemicals, present and the levels of phenolic compounds associated with insecticidal activities in those plants for the management of agricultural pests. The plant extracts were prepared using water as a solvent individually and unfermented mixture form in various proportions. Thereafter, phytochemical screening was carried out using colour indicators and Liquid chromatography–mass spectrometry (LC–MS-QTOF) to identify the present phytochemicals and their masses. The unfermented plant extracts combined with neem tested positive for alkaloids, flavonoids tannins, saponins, phenols, cardiac glycosides and terpenoids. However, terpenoids were tested in *A. sativum* and the unfermented plant extract combined with neem, while the other plant extracts tested negative for terpenoids. Using LC-MS- QTOF different types of phenolic compounds such as polyphenols, phenolic acids were tested and various groups profiles such as flavonoids and hydroxycinnamic acid in varied amounts were shown to be present. In addition, terpenes and organosulfur compounds were also detected. These bioactive compounds are associated with insecticidal activities against agricultural pests. In addition, the outcome of mixing the different aqueous plant extracts had been multiplicative rather than additive synergistic effect on the bioactive compounds as compared to the use of a single plant species. This could be beneficial in crop protection as the synergistic behavior of the bioactive compounds confers an advantage over the use of single plant extract as it can increase its efficacy against agricultural pests thus, benefiting smallholder farmers.

4.2 Introduction

Globally, productivity in food production systems is facing many challenges such as pests and diseases which are being driven by climate change (Aryal *et al.*, 2020). A wide range of synthetic pesticides have been used in the management of insect pests over time to ensure food security for the growing human population (Rani *et al.*, 2021). Despite the synthetic pesticides supporting crop production in agriculture, these pesticides are regarded as harmful to human health and the environment (Kalyabina *et al.*, 2021). The overuse and misuse of pesticides is associated with the destruction and loss of biodiversity which is very crucial to the survival of human on the planet (Tang *et al.*, 2021; Khan *et al.*, 2023). In light to these constraints, efforts to find alternative strategies that are ecologically friendly and sustainable such as the use of botanicals in the management of the insect pests are being exploited (Campos *et al.*, 2019; Lengai *et al.*, 2020).

The use of plant extracts in agriculture on management of pests has been growing over the last two decades (Pavela, 2016; Isman, 2020). The plant extracts can be prepared from plant components such as seeds, leaves, and roots for their natural bioactive compounds (Godlewska *et al.*, 2021). Bioactive compounds, which are mainly phytochemicals, are classified as primary or secondary metabolites based on the metabolic role they play in plants. Primary metabolites such as proteins, carbohydrates, vitamins, and lipids, play key roles in plant growth and reproduction (Hussein and El-Anssary, 2019). Contrary to primary metabolites, secondary metabolites play key role in defense mechanisms against microbial and insect herbivory activities in plants as reported by Pang *et al.*, 2021, which contributes to food security.

Some of the plants reported to have these secondary metabolites associated with crop protection include *A. indica, C. frutescens, A. sativum, L. camara* and *T. minuta (Oparaeke et al.*, 2005 ; Sakadzo *et al.*, 2020 ; Ujah *et al.*, 2021; Sivakumar *et al.*, 2022). These secondary metabolites are divided into major groups such as phenolic compounds, terpenoids, glycosides, alkaloids, and organosulfur compounds (Figure 4.1). The classification of these secondary metabolite is based on their biosynthetic pathways, chemical structure and properties (Lengai *et al.*, 2020; Aneklaphakij *et al.*, 2021) .

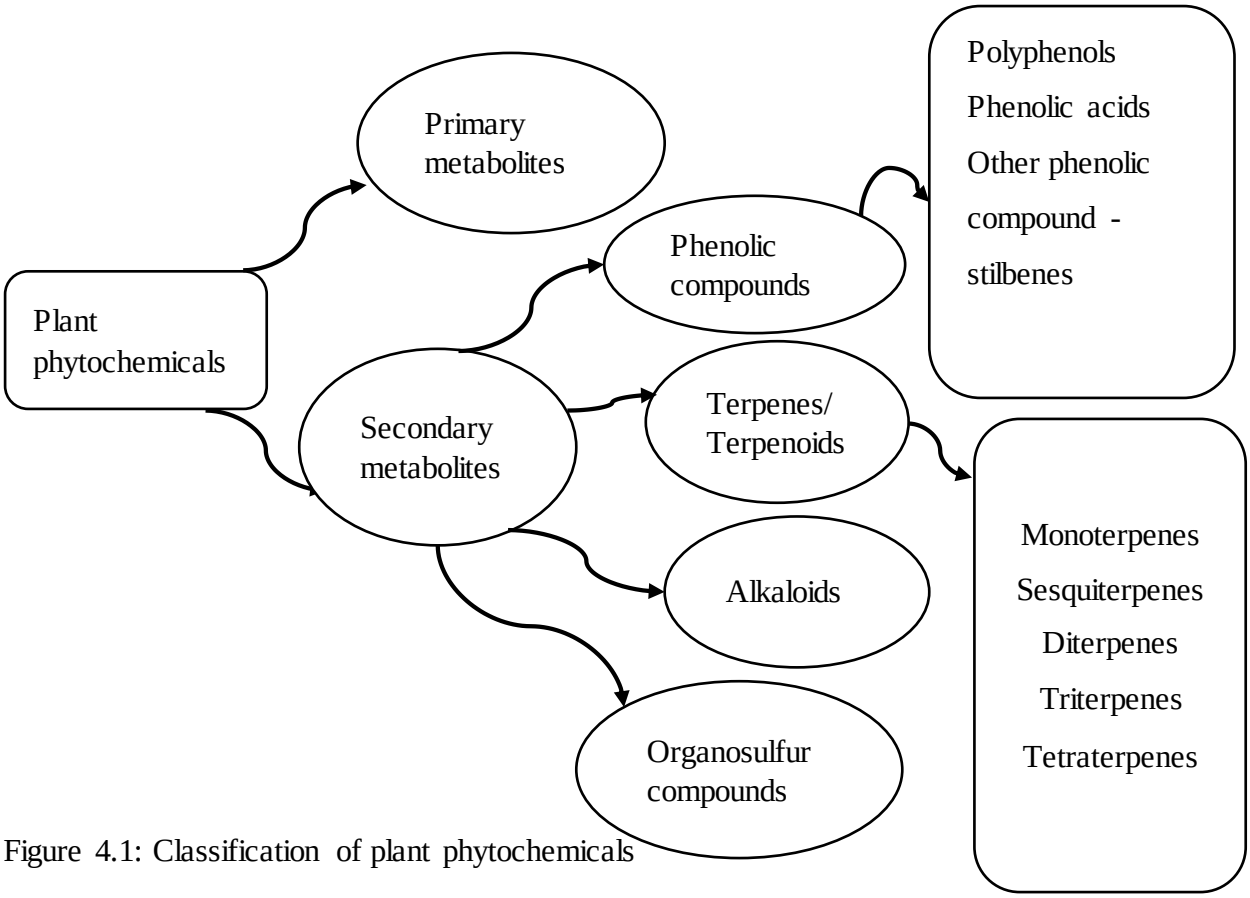

Figure 4.1: Classification of plant phytochemicals

Phenolic compounds are the main secondary metabolites produced by plants, and they can be grouped into two main classes namely simple phenolic compounds and polyphenols. These secondary metabolites have at least one phenol unit in their structure. They can be classified further into phenolic acids, flavonoids, and other phenolics such as coumarins, tannins among others (Figure 4.2) (Singla *et al.*, 2019). Flavonoid, have structural diversity due to oxidation and hydroxylation patterns of the core pyran ring (Pratyusha, 2022). The major compounds include flavanols, flavanones, isoflavones, flavones (Figure 4. 2).

They have been reported to play a key role against pathogens and insect pests in agriculture (Pang *et al.*, 2021). These bioactive compounds function in crop protection by either interfering with feeding, reproduction or altering insect pest growth and development (Lengai *et al.*, 2020). In the previous chapter (3), efficacy trials were carried out on different plants extracts, that had varied efficacies on bean flower thrips and black bean aphids. However, it was not possible to know the phytochemical compounds that were responsible on various efficacies observed against French bean thrips and black bean aphids.

Therefore, this study was designed to identify phytochemical profiles in the unfermented plant extract combined with neem that portrayed good efficacy results against French bean flower thrips and black bean aphid in laboratory, screenhouse and open field experiments. The phytochemical analysis was carried out through qualitative phytochemical screening and quantitative analysis using LC–MS QTOF with special interest to bioactive compounds with insecticidal activities.

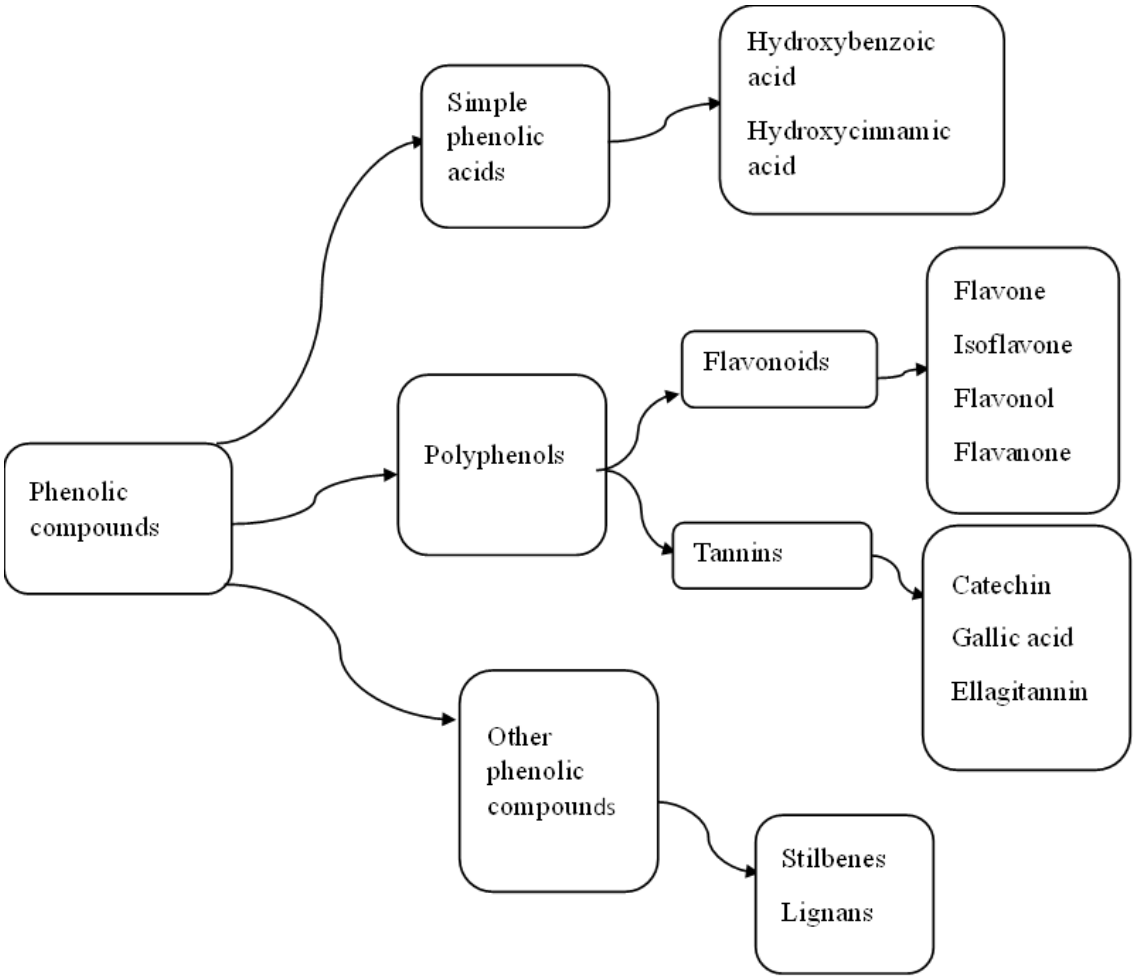

Figure 4.2: Classification of phenolic compounds

4.3*:* Materials and methods

4.3.1 Collection of plant materials

Young, fresh plant leaves/ shoots from both *Lantana camara*, and *Tagetes minuta*, were collected from the International Centre of Insect Physiology and Ecology-*icipe* compound. Mature red fruits of *Capsicum frutescens* and bulbs of *Allium sativum* were purchased from a local grocery. *Azadirachta indica* seed powder was sourced from Organic Agriculture Center of Kenya (OACK) Muran'ga, Kenya. These plant materials were brought to the laboratory at icipe where they were Leaves from *L. camara*, and *T. minuta,* fruits of *Capsicum frutescens* and bulbs of *Allium sativum* were sorted, cleaned, and thereafter, all the plant materials were weighed to prepare the various samples required for phytochemical analysis.

4.3.2 The choice of the plant extract samples for analysis

The choice of samples to be analyzed was guided by the best performing plant extracts in the management of bean flower thrips and black bean aphid on French beans crop in the previous chapter (3). The unfermented plant extract combined with neem (UPE +N) gave best efficacy results and was selected as a sample for analysis besides, phytochemical profile analysis of the individual plant extracts. This gave rise to a total of seven samples to be analyzed which were as follows; *Tagetes minuta, Lantana camara, Capsicum frutescens, Allium sativum, Azadirachta indica* and a mixture of all the 5 plant extracts (UPE +N), and tap water was used as a negative control.

4.3.3 Plant extract preparations for phytochemical analysis

The plant extracts samples were prepared as described in (Table 4.1). The resulting plant extract solutions were used on phytochemical screening using qualitative protocols. and thereafter quantitatively analyzed using LC-MS QTOF to identify different phytochemical profiles in the plant extracts.

Table 4.1: Preparation of the plant extracts samples for the phytochemical analysis

Plant sample	Preparation methods
Tagetes minuta	Hundred grams of *T. minuta* young leaves were cut into small pieces of an average size of 0.5 cm. Five hundred milliliters of tap water was added and the mixture soaked for 24 hours. The solution was sieved to obtain *T. minuta* extract.
Lantana camara	Hundred grams of *L. camara* young leaves were cut to small pieces of an average size of 0.5 cm. Five hundred milliliters of tap water was added and the mixture soaked for 24 hours. The solution was sieved to obtain *L. camara* extract.
Capsicum frutescens	Fifty grams of *C. frutescens* mature red fruits were crushed to a fine paste. Five hundred milliliters of tap water were added, and the mixture soaked for 24 hours. The solution was sieved to obtain *C. frutescens* extract.
Allium sativum	Fifty grams of *A. sativum* bulbs were crushed to a fine paste, five hundred milliliters of tap water were added, and the mixture was soaked for 24 hours. The solution sieved was to obtain *A. sativum* extract.
Azadirachta indica	Twenty-five grams of *A. indica* seed powder was weighed, and added into five hundred milliliters of tap water and the mixture was soaked for 24 hours. The solution was sieved to obtain *A. indica* solution.
Unfermented plant extracts (UPE + N)	A hundred grams of *T. minuta and L. camara* each, fifty grams of *C. frutescens* and *A. sativum* each, and twenty-five grams of *A. indica* were prepared as above. The mixture was soaked in five hundred milliliters of tap water for 24 hours, the mixture was thereafter sieved to obtain a solution of mixed plant extracts (UPE + N).

4.3.4 Qualitative phytochemical screening of different plant extracts

The phytochemical screening of the plant extracts was done using standard procedures to determine the presence or absence of some selected secondary metabolites using the protocol described by (Gul *et al.*, 2017; Virshette *et al.*, 2019; Siddiqui, 2021). Tests carried out were based on colour changes caused by the interaction between bioactive compounds and specific chemicals. The secondary metabolites of interest were classified as polyphenols which included flavonoids, tannins, and phenolic acid, and the other category of phytochemicals of interests was terpenoids that included saponins and cardiac glycosides. In addition, alkaloids were also tested in the aqueous plant extracts.

An alkaline reagent test was carried out to determine the presence of flavonoids in the plant extracts. On this test two millilitre of different aqueous plant extract solutions were mixed with 2 % Sodium hydroxide separately. Production of a yellow colour, which turned colourless after adding two drops each at 200 µl of dilute 0.1 M Hydrochloric acid indicated the presence of flavonoids. The test for tannin was carried out by drawing 2 ml of each plant extract sample into 15 ml falcon tubes. Thereafter, two drops approximately 200 µl of ferric chloride was added to the mixture. The formation of blue-black or blue-green colour indicated the presence of tannins (Khanal, 2021).

Terpenoids were tested in different plant extract samples by picking 2 ml of plant extract into a 25 ml falcon tube and adding 2 ml chloroform analytical grade (Sigma Aldrich). This was followed by slowly addition of 2 ml concentrated H_2SO_4 and heating the mixture for 2 minutes in a water bath. The presence of brown-reddish colour indicated positive results for terpenoids. Further tests on two key groups of terpenoids (Saponins and Cardiac glycosides) were also carried out. To determine the presence of cardiac glycosides, Salkowski's test was carried out, where 2 ml of different plant extract samples were treated with 2 ml chloroform, followed by addition of 2 ml concentrated H_2SO_4 that was added slowly to the mixture. The presence of brownish, reddish colour indicated presence of cardiac glycosides (Panchal and Parvez, 2019). Saponin tests was carried out by shaking vigorously 2 ml of plant extract and development of a stable froth/foam indicated the presence of saponins in the sample(Gul *et al.*, 2017). The test for alkaloids was also done using 2 ml of each plant extract sample and adding 5 ml, 1.5 % HCl (v/v) followed Meyer's reagent into the mixture. The appearance of turbidity or any changes in colour to yellowish indicated the presence of alkaloids (Shaikhand Patil, 2020).

4.3.5 Quantitative phytochemical analysis of aqueous plant extracts using LC-MS QTOF

Individual aqueous plant extracts from five plants namely, *Azadirachta indica*, *Capsicum frutescens, Allium sativum, Lantana camara*, and *Tagetes minuta* were analyzed. A mixed aqueous plant extract was also prepared using all the five plants and analyzed. These plant extracts samples were screened and analysed for various phytochemicals present. All the samples were prepared in tap water through soaking for 24 hours, followed by sieving to remove large debris, and thereafter centrifugation at 4000 rpm for 5 minutes at room temperature. Using a micropipette, 200 µl of the supernatant was transferred into 2 ml clear glass vials (Supelco, Bellefonte, PA, USA) each containing 800 µl distilled deionized tap water and immediately analysed using LC-MS QTOF. All the analyses were carried out in triplicates using different batches of plant materials. The chromatographic separation was achieved on an Agilent system 1100 series (MA, USA) using ZORBAX SB-C18, 4.6 × 250 mm, 3.5 µm column, operated at 40 °C. Mobile phases used were made up of tap water (A) and acetonitrile (B) each with 0.01 % formic. The following gradient was used: 0 – 8 min, 10 % B; 8 – 14 min, 10 – 100 % B; 14 – 19 min, 100 % B; 19 – 21 min, 100 – 10 % B; 21 – 25 min, 10 % B. The flow rate was held constant at 0.5 ml min^{-1} and the injection volume was 10 µl. The LC was interfaced with a quadrupole mass spectrometer. The mass spectrometer was operated on ESI-positive mode at a mass range of m/z 50–600 at 70 eV cone voltage Flavonoids (kaempferol, quercetin and luteolin), caffeic acid and proline of the authentic standards from Sigma–Aldrich, St. Louis, MO, USA were prepared in methanol at 100 $\mu g kg^{-1}$ and scanned to generate a library of spectra for comparison with spectra obtained from analyzed sample (Majors and Wilmington, 2013).

4.4. Results

4.4.1 Qualitative phytochemical screening of different aqueous plant extracts

The phytochemical screening of unfermented plant extract combined with neem revealed the presence (+) of alkaloids, flavonoids, tannin, saponin, phenolic, cardiac glycoside and terpenoid. Alkaloids and saponin were present in all tested plant extracts except in *A. indica*. On the other hand, flavonoid was present in all tested plant extracts, except in *A. sativum*. However, terpenoids were absent in the other three plant extracts except in *A. indica, A. sativum* and in unfermented plant extract combined with neem (UPE +N) (Table 4.2).

Table 4.2: The presence of phytochemicals in aqueous plant extracts

Phytochemical	Aqueous plant extracts samples					
	L. camara	*T. minuta*	*A. indica*	*C. frutescens*	*A. sativum*	UPE +N
Alkaloid	+	+	-	+	+	+
Flavonoid	+	+	+	+	-	+
Tannin	+	+	-	+	-	+
Saponin	+	+	-	+	+	+
Phenolic	+	+	-	-	-	+
Cardiac glycoside	+	+	-	-	-	+
Terpenoid	-	-	+	-	+	+

Where (+) = presence while (-) = absence

4.4.2 Quantitative phytochemical analysis of aqueous plant extracts using LC-MS QTOF

A total of 24 major phytochemicals profiles of the aqueous plant extracts were detected using LC-MS QTOF (Figure 4.2 and Table 4.3). Polyphenols formed a major composition of the phytochemicals of importance in crop protection found in the plant extracts. Of the polyphenol detected, flavonoids, phenolic acids, and coumarins formed the major composition. In all plant extracts, different groups of flavonoids such as flavones, flavanols, flavan-3-ols and flavanones were detected. Different types of flavonol such as quercetin, rutin and kaempferol were found to be present in all plant extracts in different concentration. In the aqueous unfermented plant extract combined with neem, the concentration of flavonol such as rutin and kaempferol was higher compared to their concentration in individual plant extracts.

Phenolic acids such as hydroxycinnamic and hydroxybenzoic acids were also present in all the plant extracts. Their concentrations were higher in the unfermented plant extract combined with neem compared to each individual plant extract just like in the case of flavonol. Terpenes such as lantaninilic acid were detected in *L. camara* extract and in unfermented plant extract combined with neem. The concentration of lantaninilic acid unfermented plant extract combined with neem was high as compared to the concentration in *L. camara*. S-allyl-cysteine, which is an organosulfur, was not detected in majority plant extracts except in *A. sativum* (Table 4.3).

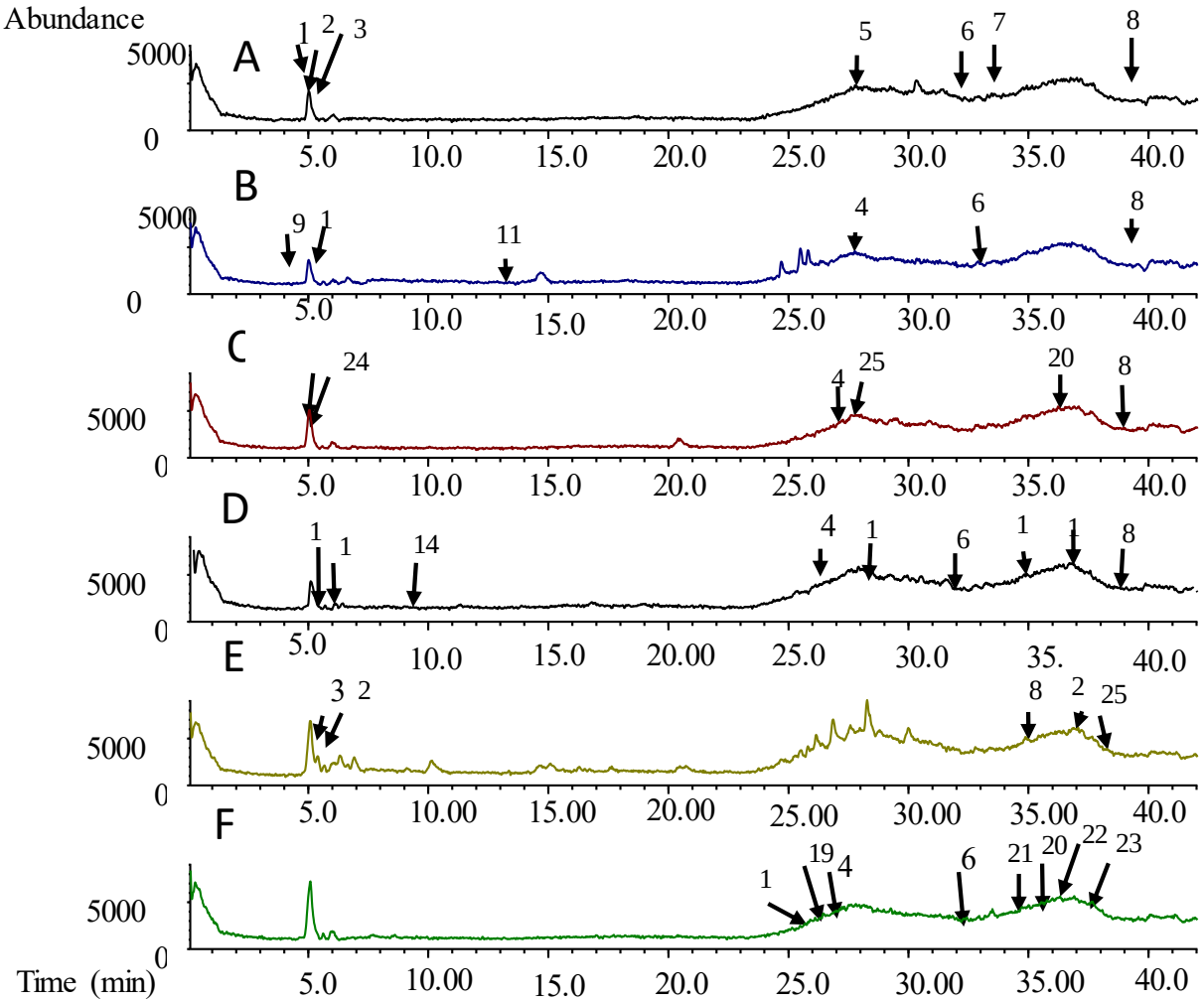

Figure 4. 3: Chromatograms from plant extracts separated using LC-MS QTOF. Where A- *C. frutescens*, B- *A. sativum*, C- *L. camara*, D- *A. indica*, E- UPE +N

Table 4.3: Characterization of major phenolic, terpenes and organosulfur compounds in aqueous plant extracts by LC-MS QTOF

Plant sample	Retention time (Min)	Compound name	Compound group	Molecular weight (amu)	Observed mass-to-charge ratio $(M+H)^+$	Concentration (pg /µl)
Capsicum frutescens	5.0	Vanillin	Hydroxybenzoic acid	152.2	153.1	51.4
	5.0	4-Hydroxybenzoic acid	Hydroxybenzoic acid	138.1	139.1	27.9
	5.1	Caffeic acid	Hydroxycinnamic acids	180.2	181.0	57.7
	12.0	Gallic acid	Hydroxybenzoic acid	170.1	170.1	29.2
	27.8	Luteolin	Flavones	286.2	287.6	62.1
	33.5	Quercetin	Flavonol	302.2	303.0	6.7
	35.9	Oleuropein	Other phenolics	540.5	541.1	48.0
	40.5	Rutin	Flavonol	610.5	611.2	14.9
Allium sativum	5.0	S-allyl-cysteine	Organosulfur	161.2	162.3	16.3
	13.4	Pyrogallol	Hydroxybenzoic acid	126.1	127.3	60.2
	27.7	Gallic acid	Hydroxybenzoic acid	170.1	171.1	68.5
	33.3	Quercetin	Flavonol	302.2	303.1	27.3
	40.5	Rutin	Flavonol	610.5	611.2	14.9
Azadirachta indica	5.8	(+)-Catechin	Flavan-3-ols	290.3	291.8	19.2
	5.8	(-)-Epicatechin	Flavan-3-ols	290.3	291.8	15.1
	11.4	Nimbidiol		274.4	275.0	8.3
	27.7	Gallic acid	Hydroxybenzoic acid	170.1	171.1	11.6
	29.4	Nimbandiol		456.5	457.2	3.8
	33.9	Quercetin	Flavonol	302.2	303.4	21.1
	36.8	Margolonone		314.4	337.1	4.2
	38.6	Kaempferol 3-glucoside	Flavonol	447.9	448.5	3.0
	40.5	Rutin	Flavonol	610.5	611.9	2.2

Conti…Table 4.3: Characterization of major phenolic, terpenes and organosulfur compounds in aqueous plant extracts by LC-MS QTOF

Plant sample	Retention time (Min)	Compound name	Compound group	Molecular weight (amu)	Observed mass-to-charge ratio $(M+H)^+$	Concentration (pg/µl)
Tagetes minuta	26.9	Naringenin	Flavanones	272.3	273.1	21.1
	27.5	Apigenin	Flavones	270.2	271.0	20.4
	28.4	Gallic acid	Hydroxybenzoic acid	170.1	171.2	21.7
	33.4	Quercetin	Flavonol	302.2	303.4	87.7
	35.7	Kaempferol	Flavonol	286.2	287.6	17.2
	36.3	Naringin	Flavanones	580.5	581.1	7.5
	36.4	Luteolin	Flavones	286.2	287.2	39.7
	37.1	Kaempferol 7-O-glucoside	Flavonol	447.9	448.3	83.5
Lantana camara	5.0	Caffeic acid	Hydroxybenzoic acid	180.2	181.0	30.3
	5.1	p-Coumaric acid	Hydroxycinnamic acids	164.1	165.0	191.9
	27.8	Gallic acid	Hydroxybenzoic acid	170.1	171.1	52.9
	28.9	Lantaninilic acid	Terpenes	486.7	487.2	60.6
	36.1	Kaempferol	Flavonol	286.2	287.1	17.4
	40.5	Rutin	Flavonol	610.5	611.7	154.2
Unfermented plant extracts combined with neem	5.0	Caffeic acid	Hydroxycinnamic acids	180.2	181.8	177.7
	5.1	4-Hydroxybenzoic acid	Hydroxybenzoic acid	138.1	139.0	770.6
	36.3	Rutin	Flavonol	610.5	611.7	176.0
	37.7	Kaempferol	Flavonol	286.2	287.1	328.0
	38.3	Lantaninilic acid	Terpenes	486.7	487.5	750.5

4.4.3 The diversity of chemical structures of major phytochemicals compounds found in different aqueous plant extracts

All the chemical structures of the phytochemicals detected in the plant extracts had a common basic structure composed of a hydroxyl group attached to a benzene ring which is a characteristic of polyphenols. Phenolic acids, which comprise cinnamic acid and benzoic acid derivatives were based on C3–C6 and C1–C6 backbones such as vanillin, 4-hydroxybenzoic acid, gallic acid, and caffeic acid. A general backbone structure of C6-C3-C6 was exhibited by flavonoids. The two C6 units are usually phenolic, and they are categorised further into different sub-groups such as flavanones, flavonol, flavones and flavan-3-ols based on hydroxylation patterns and differences in the chromane ring (C3). The common flavonol to all plant extracts were kaempferol, quercetin and their glycosidic derivatives (Figures 4.4 to 4.9).

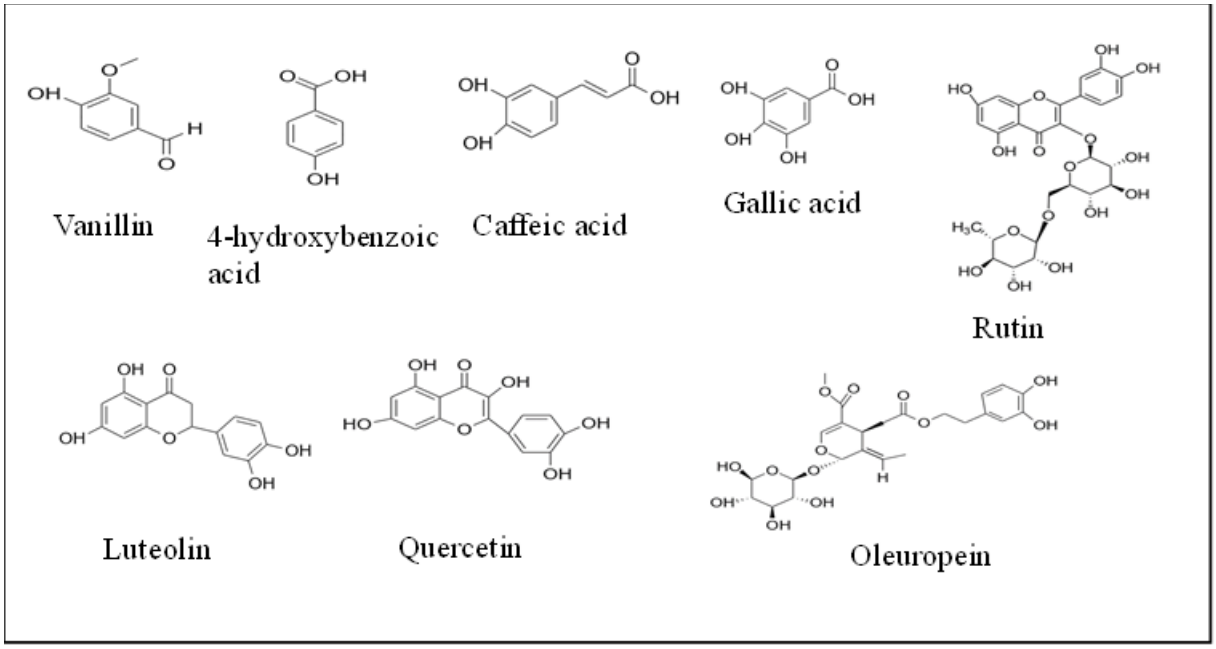

Figure 4.4: Major chemical structures of phytochemicals in *Capsicum frutescens*

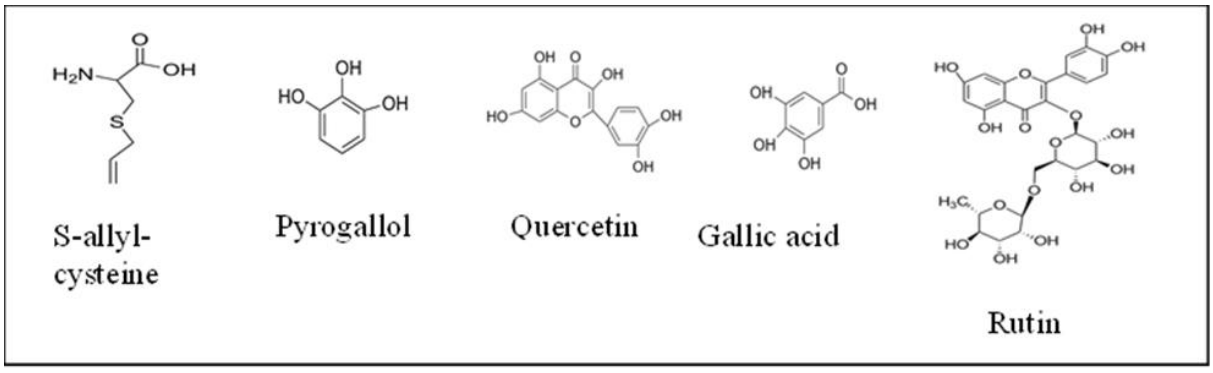

Figure 4.5: Major chemical structures of phytochemicals in *Allium sativum*

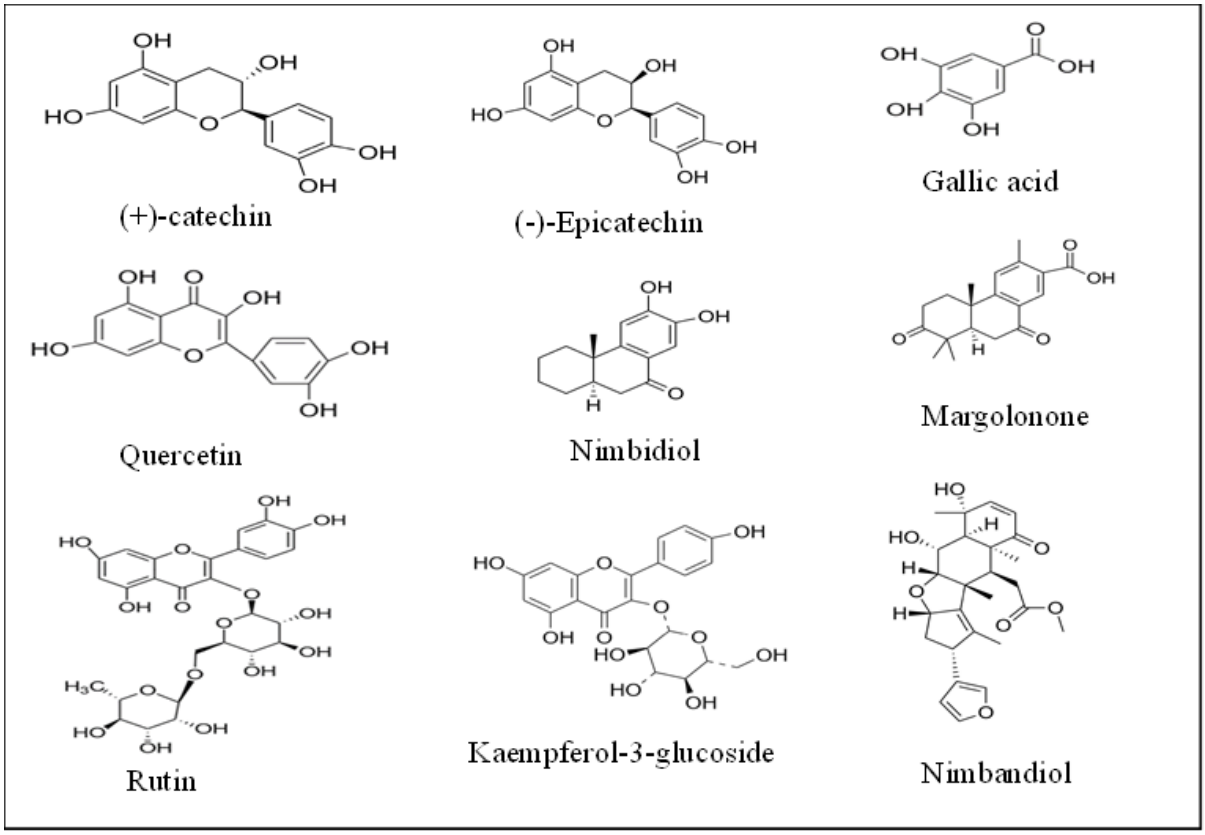

Figure 4.6: Major chemical structures of phytochemicals in *Azadirachta indica*

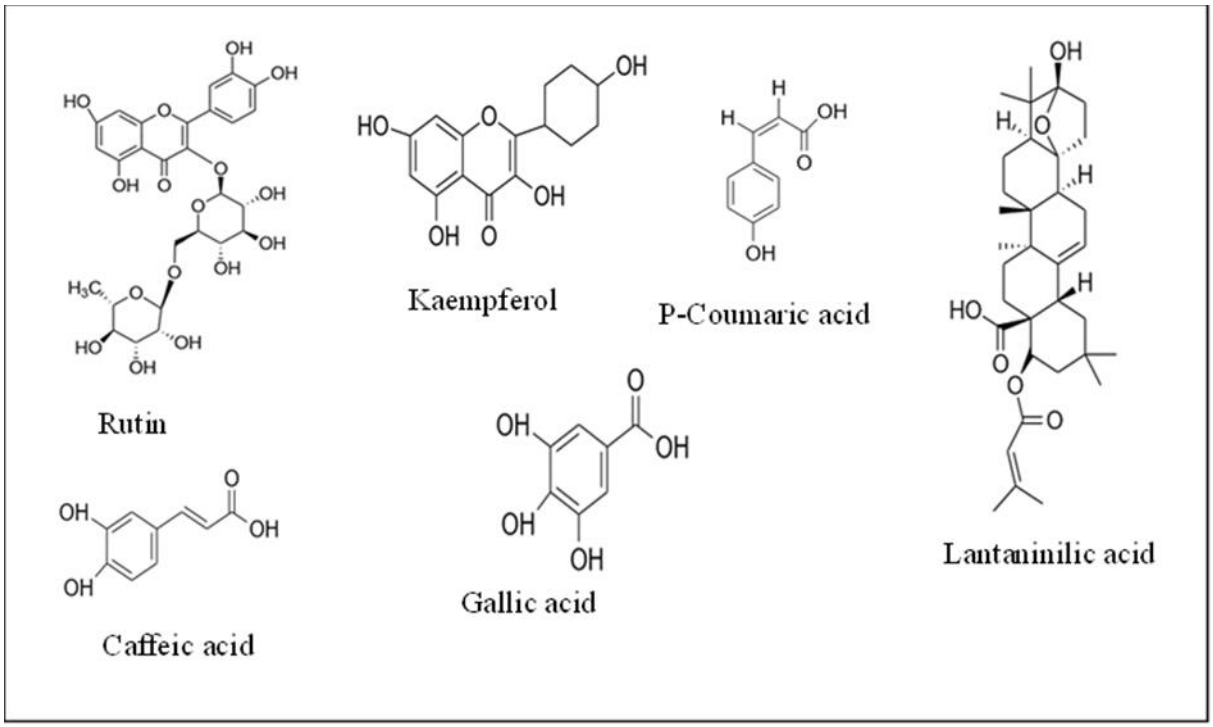

Figure 4.7: Major chemical structures of phytochemicals in *Lantana camara*

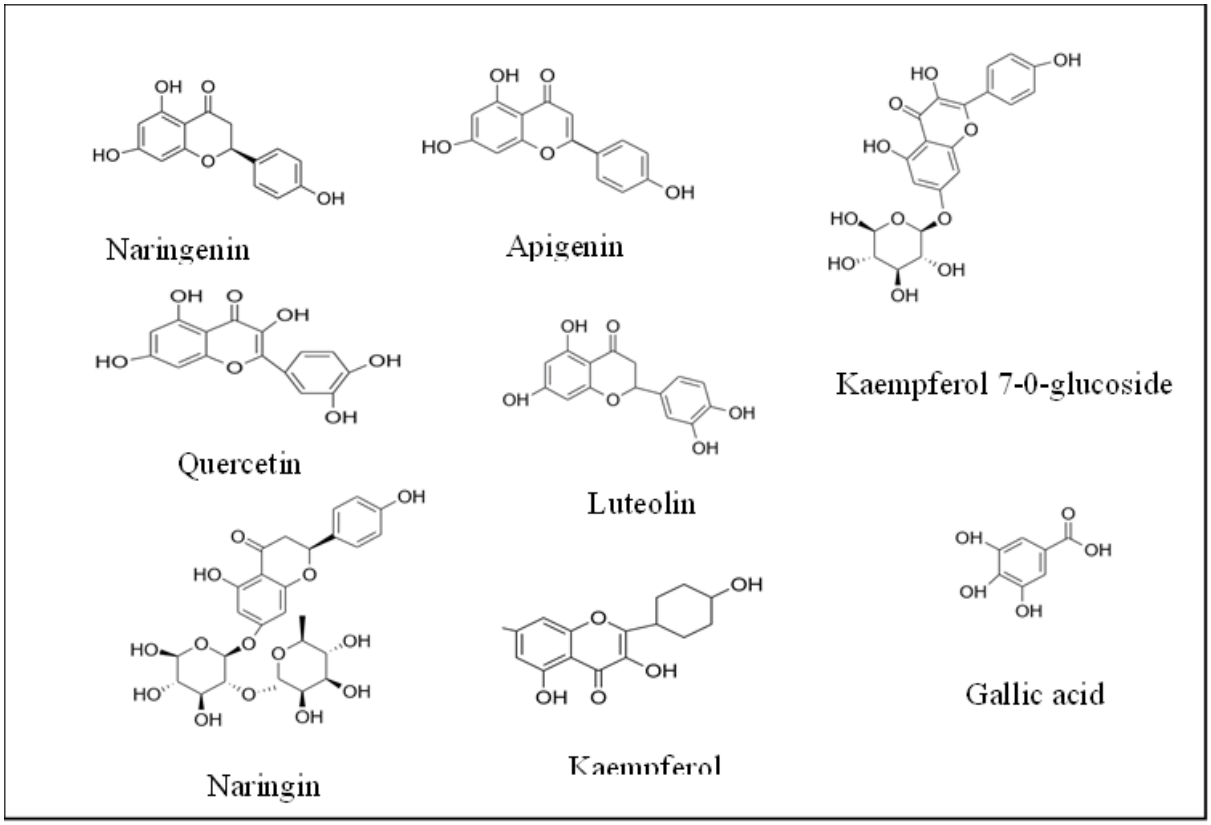

Figure 4.8: Major chemical structures of phytochemicals in *Tagetes minuta*

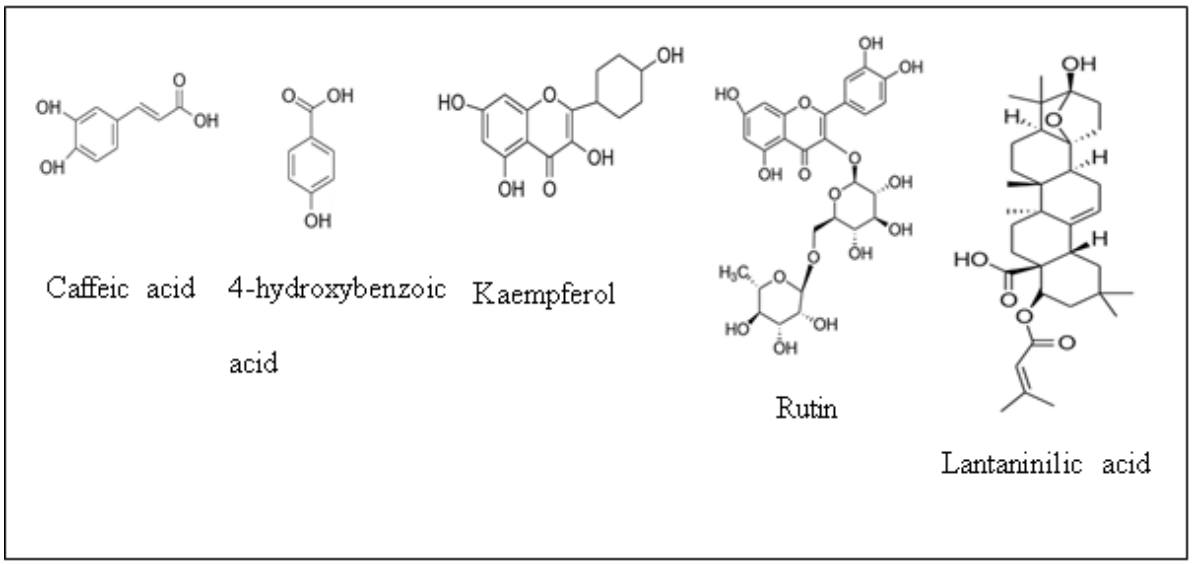

Figure 4.9: Major chemical structures in unfermented plant extract combined with neem powder

4.5 Discussion

The phytochemical screening of aqueous plant extracts from the fruits of *Capsicum frutescens,* bulb of *Allium sativum,* seedcake powder of *Azadirachta indica* and leaves of *Lantana camara,* and *Tagetes minuta* revealed the presence of diverse categories of phytochemicals. These diverse categories of phytochemicals included alkaloids, flavonoids tannins, saponins, phenols, cardiac glycosides and terpenoids. The results from the current study concurred with previous studies which reported presence of tannin, flavonoids, alkaloids and terpenoids in *T. minuta* , *A. indica* and *C. frutescens* (Hadjiakhoondi *et al.*, 2005; Palma-Tenango *et al.*, 2017; Lamara *et al.*, 2020). While terpenoid were reported in *A. indica* and *C. frutescens* (Diabaté *et al.*, 2020; Rosulu *et al.*, 2022). The various categories of phytochemical were further analysed using LC-MS to identify the specific compounds and their masses to charge ratio.

The LC-MS analysis revealed that the various plant extracts had diverse groups of major phytochemicals with varied masses to charge ratio. This could have been attributed to different types of plants from different families, growing zones, and growth stages of the plant (Howard *et al.*, 2000; Hazrati *et al.*, 2020 ; Muhizi *et al.*, 2021). On mixing the various aqueous plant extracts the effect on bioactive compounds was multiplicative rather than additive. The multiplicative effect of bioactive compounds was observed in the unfermented plant extract combined with neem, where the concentration of bioactive compounds such as, kaempferol, lantaninilic acid, rutin, caffeic acid and 4-hydroxybenzoic acid were multiplicative in the rather than additive when compared to their concentrations individual plant extracts. The multiplicative and synergistic effects of mixing plant extracts, may enhance the efficacy of the bioactive compounds against insect pests and confer an advantage over the use of single plant extracts in the management of agricultural pests (Ajayi *et al.*, 2020).

The phytochemicals identified in this study have been associated to important properties such insect repellence, larvicidal effects, growth regulation and oviposition deterrent which can play a significant role in the management agricultural pests in farms. Tannins which are bitter polyphenols inhibit feeding in phytophagous insects (El-Aswad *et al.*, 2023). They interfere with insects feeding behaviour by acting on peripheral sensilla (Isman, 2002 ; Chaitanya, 2017). In this study, triterpenes such as saponins, lantaninilic acids and cardiac glycosides were present in the plant extracts. Previous studies have revealed that saponins, which are glycosylated triterpenes have insecticidal effects against insect pests (Zaynab *et al.*, 2021).

These phytochemicals also have different chemical structures and properties which enables them to have different mode of action against the target pests could enhance their effectiveness (Kim *et al.*, 2021).

Saponins have been reported by Chaieb, 2010 to have insecticidal effects against pests. They interact with cholesterol molecules in prothoracic glands of the insect, inhibiting production of ecdysteroid which is a hormone responsible for molting thus interfering with growth and development of the insects (Singh and Kaur, 2018). Saponins have also been reported to repel insect pests. They interfere with the peripheral nervous system of insect causing them not to feed leading to starvation and death of insect (Boate and Abalis, 2020). In this study, the presence of saponin in the plant extracts such as *L. camara, T. minuta, A. sativum, C. frutescens* and in the unfermented plant extracts combined with neem (UPE + N) can be useful in agricultural pest management.

Flavonoids have been reported to inhibit enzymatic activity of glutathione S-transferase Noppera-bo (Nobo) which is responsible for biosynthesis ecdysone hormone during immature stages of insects thus interfering with their growth and development. Rutin, luteolin and quercetin interferes with the moulting and reproduction processes of several insects by inhibiting the formation of ecdysone and juvenile hormone (Oberdörster *et al.*, 2001). Quercetin, rutin, and naringin were found to be effective in controlling the nymphs and adults stages of aphid (Goławska *et al.*, 2014;- Palma-Tenango *et al.*, 2017). The diverse modes of action against agricultural pests exhibited by flavonoids, makes them a suitable candidate to be used in crop protection especially in pests' management. Though it is important to carry out more studies to ascertain their dose concentration with insecticidal effect.

Phenolic acids which are compounds with one aromatic ring attached to a single carboxylic acid functional group such as hydroxybenzoic and hydroxycinnamic acid (Singla *et al.*, 2019) were also identified in the various plant extracts. In previous studies, vanillic acid and *4*-hydroxybenzoic acid in *C. frutescens* were reported to inhibit acetyl-cholinesterase in rice weevils (Singh *et al.*, 2021). In this study they were present in all plant extracts in varies masses to charge ratio, and hence the positive efficacy results exhibited by plant extracts making them ideal candidates in agricultural pest management strategies.

4.6 Conclusion

The phytochemical analysis of the aqueous plant extracts demonstrated the presence of secondary metabolites known for their insecticidal properties. It was observed that combining different aqueous plant extracts resulted in a synergistic effect, amplifying the bioactive compounds' effectiveness beyond mere additive effects. This synergistic interaction holds promising potential for enhancing the pest management capabilities using mixed plant extracts. Smallholder farmers stand to benefit from this approach as it surpasses the efficacy of using a single plant in extract preparation. Nonetheless, further investigations should be conducted to isolate and examine specific compounds, both individually and in combination, to gain a deeper understanding of their impact on insect behavior. These studies will contribute to advancing our knowledge and fine-tuning strategies for pest control using plant extracts in agricultural settings.

CHAPTER FIVE: GENERAL DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

5.1 Discussion

The efficacy results in the laboratory, screenhouse and field experiments from this study, revealed the potential of aqueous unfermented plant extracts combined with neem in the management of the bean flower thrips and black bean aphid in French beans. The different plant extracts investigated had varied ability to induce mortality on the bean flower thrips and black bean aphid across various concentrations in different treatments. This could be attributed to their different combination of bioactive compounds and their different preparation method (Tavares *et al.*, 2021; Rajagopal *et al.*, 2022). Some of the plant extracts were prepared through fermentation process for a period of 14 days while others were extracted and used after 24 hours. The percent mortality induced on French bean thrips and aphids by different concentrations of fermented plant extracts at any concentration was below 50 %. This could be attributed to the decomposition of bioactive compounds through hydrolysis reactions as a result of environmental factors (Lefebvre *et al.*, 2021).

The unfermented plant extract combined with neem (UPE + N) showed promising results when compared to the use of single plant extracts in the management of bean flower thrips and black bean aphids. The UPE + N contained *Lantana camara, Tagetes minuta, Azadirachta indica, Capsicum frutescens* and *Allium sativum* plant materials and all these plants are known to have varied bioactive compounds with insecticidal activities. The bioactive compounds in neem are azadirachtin, nimbidol, nimbolinin, quercetin, and nimbin. These compounds are known to be repellent, antifeedant, interfere with reproduction and feeding of insects (Liang *et al.*, 2003; Sow *et al.*, 2015; Ghoneim *et al.*, 2017).

Azadirachtin, which is classified under terpenoids is a complex bioactive compound with anti-insecticidal effects, and it has a structure which resembles that of ecdysone hormone which is responsible for insect metamorphosis (Kilani *et al.*, 2021). This enables the bioactive compound to bind to the insect receptors such as taste receptors in the mouthparts. The signal is encoded in the central nervous system thus sending signals to stimulate the chemoreceptors especially the deterrent cells to block feeding stimulation in the insect (Chaudhary *et al.*, 2017). Likewise , *L. camara*, contains triterpenoids and lantadane which have antifeedant activities (Dua *et al.*, 2010). *Tagetes minuta* has been reported to have repellent effects on insects (Phoofolo, 2013).

C. frutescens has been documented to have larvicidal effects on insects pests such as mosquito larvae (Parmar and Amit Gangwal, 2011) while *A. sativum* has been reported to have to have insecticidal effects on insects such as coleoptera (Dougoud *et al.*, 2019). Therefore, these multiple modes of action have synergistic effect and could also have contributed to the observed efficacy of UPE + N against thrips and aphids on French beans even though the exact mode of action was not evaluated in this study.

The phytochemical screening of aqueous plant extracts from the fruits of *Capsicum frutescens,* bulb of *Allium sativum,* seedcake powder of *Azadirachta indica* and leaves of *Lantana camara,* and *Tagetes minuta* revealed the presence of diverse categories of phytochemicals. These diverse categories of phytochemicals included alkaloids, flavonoids, tannins, saponins, phenols, cardiac glycosides and terpenoids. Results from this study concurred with previous studies which reported presence of tannin, flavonoids, alkaloids and terpenoids in *T. minuta* , *A. indica* and *C. frutescens* (Hadjiakhoondi *et al.*, 2005; Palma-Tenango *et al.*, 2017; Lamara *et al.*, 2020), and terpenoid in *A. indica* and *C. frutescens* (Diabaté *et al.*, 2020; Rosulu *et al.*, 2022).

Polyphenols are known to have antifeedant properties to phytophagous insect. They interferes with insects feeding behaviour by acting on peripheral sensilla (Chaitanya, 2017; Isman, 2002). Tannins also play a major role on inhibiting feeding in insects (Wang *et al.*, 2023). They interfere with protein digestion, thus affecting insects' development.

Triterpenes such as saponins, lantaninilic acids cardiac glycosides were also present in the unfermented mixed plant extract combined with neem. These compounds are associated with antifeedant and deterrent activities on insect pests and this could explain the observed effectiveness of unfermented mixed plant extract combined with neem against bean flower thrips and black bean aphid on French beans in all experiments. Previous studies have shown that saponins, which are glycosylated triterpenes act as feeding deterrents against insect pests (Thimmappa *et al.*, 2014). Saponins have been reported by Chaieb, 2010 to have insecticidal effects against pests. They interact with cholesterol molecules in prothoracic glands of the insect, inhibiting production of ecdysteroid which is a hormone responsible for molting thus interfering with growth and development of the insect (Singh and Kaur, 2018). Saponins have also been reported to repel insect pests. They interfere with the peripheral nervous system of insect causing them not to feed leading to starvation and death of the insect (Boate and Abalis, 2020).

Flavonoids were also present in plant extracts, and they have been reported to inhibit enzymatic activity of glutathione S-transferase Noppera-bo (Nobo) which is responsible of biosynthesis ecdysone hormone during immature stages of insects thus interfering with their growth and development. Rutin, luteolin and quercetin interferes with the molting and reproduction processes of several insects by inhibiting the formation of ecdysone and juvenile hormone (Oberdörster *et al.*, 2001). Quercetin, rutin, and naringin were found to be effective in controlling the nymphs and adults stages of aphid (Goławska *et al.*, 2014;- Palma-Tenango *et al.*, 2017). The diverse modes of action against agricultural pests exhibited by flavonoids, makes them a suitable candidate to be used in crop protection especially in pests' management.

Phenolic acids which are compounds with one aromatic ring attached to a single carboxylic acid functional group such as hydroxybenzoic and hydroxycinnamic acid (Singla *et al.*, 2019) were also identified in the various plant extracts. In previous studies, vanillic acid and *4*-hydroxybenzoic acid in *C. frutescens* were reported to inhibit acetyl-cholinesterase in rice weevils (Singh *et al.*, 2021). Phenolics have also been documented by Ninkuu *et al.* (2021) to cause delayed maturation of *Aphis gossypii* while lowering their fecundity on cotton.

The compounding effects of these bioactive compounds could have negative effects on the insect pests hence high percentage mortalities experienced on bean flower thrips and black bean aphid. Therefore, the diverse mode of action of these bioactive compounds could have contributed to the high efficacy of the unfermented mixed plant extracts combined with neem. The current study has demonstrated that, when bioactive compounds interact, the outcome can be synergistic. The outcome from interaction of bioactive compounds in the mixed unfermented plant extract was multiplicative rather than additive when compared to bioactive compounds in single plant extract. These could explain the efficacy of the mixed unfermented plant extracts compared to the individual plant extracts on bean flower thrips and black bean aphid. This phenomenal, was observed by (García-Pérez, 2016) in previous studies and it could be beneficial in crop protection as aqueous mixed plant extracts, can be incorporated in pest management strategies. The current findings give a platform for future research, and exploration of mixed plant extracts for pest management.

5.2 Conclusion

The unfermented plant extract combined with neem was effective against bean flower thrips and black bean aphid. The phytochemical analysis of this plant extract revealed secondary metabolites which have been reported to have insecticidal effects in previous studies. It was also observed that combining different aqueous plant extracts resulted in a synergistic effect, amplifying the bioactive compounds' effectiveness beyond mere additive effects. Thus, the synergistic effects of bioactive compounds can contribute to pest management by the small-scale farmers at farm level. Lastly, the process of preparing these plant extracts is time consuming but the benefits towards human, animal and environmental health supersede the time and energy invested during the preparation processes. The production process is also sustainable and can be done at farm level giving commercial biopesticides and synthetic pesticides a competitive advantage.

5.3 Recommendations

Our current study has shown that, the mixed unfermented plant extract can be used in the management of bean flower thrips and black bean aphid on French beans. I therefore recommend unfermented plant extract combined with neem as an alternative plant based biopesticide for use by farmers during French bean production against thrips and aphids. In addition, more studies need to be carried out to determine the detailed effects/ modes of action associated with various bioactive compound on the target insect pests

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