THE ROLE OF FIELD MARGIN VEGETATION AND NATURAL ENEMIES IN THE MANAGEMENT OF BEAN APHIDS ON DOLICHOS BEAN (*Lablab purpureus* L.)

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DECLARATION AND RECOMMENDATION

Declaration

I hereby declare that this is my original work and has not been presented for examination in this or any other university.

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Recommendation

This thesis has been submitted with our approval as university supervisors.

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DEDICATION

I dedicate this work to the Almighty God who has been a faithful provider for all my needs throughout the study period. To my parents Mr. J.O Maragia and Mrs. B.M Obanyi for consistently encouraging me to keep studying to higher levels. My husband Mr. I.M Nyaega, son Brian, daugthers Lindah, Brendah and Belindah for their immense understanding and support in all possible ways during the entire study duration.

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ABSTRACT

Bean aphid (*Aphis fabae*) is a major pest of dolichos bean (*Lablab purpureus* L.) in Kenya. The over-reliance on synthetic insecticides to manage this insect pest poses serious risks to human health and the environment. Use of natural enemies in biological control provides a sustainable option of controlling aphids in dolichos. However, lack of diversity and abundance of natural enemies is a key constraint in effecting biological control. There is need for conservation of natural enemies to increase their diversity and abundance and enhance their ability to regulate pest populations below economic threshold levels. The objectives of this study were to determine the; i) effect of diverse field margin vegetation and cropping systems on bean aphids and their natural enemies in dolichos, ii) effectiveness of planted field margin vegetation species in supporting natural enemy populations and reducing bean aphid damage in dolichos and iii) diversity and abundance of floral resources that support natural enemies outside the main crop growing season. Field and cage exclusion experiments were conducted to evaluate the effect of field margin vegetation in conserving and enhancing the diversity and abundance of bean aphid natural enemies. The on-farm experiments were conducted in two agro-ecological zones: Njoro (LH_3) and Rongai (LH_4) while the on-station trials were carried at the Agronomy Field Station, Egerton University, during the 2019 and 2020 cropping seasons. Results from on station trials showed that maize-dolichos intercrop had 15.5% lower abundance of natural enemies than the lablab monocrop. Dolichos monocrop showed a higher bean aphid abundance 54.4, incidence 27.1% and severity of damage 9.1% compared to doichos-maize intercrop at 26.0, 15.6% and 5.9% for aphid abundance, incidence and severity of damage, respectively. On farmer's fields dolichos monocrop, a higher grain yield was observed in 2019 (2.8 tha^{-1}) compared to 2020 (2.0 tha^{-1}). Similarly, for maize-dolichos intercrop a higher land equivalent ratio (LER) of 1.27 was observed in 2019 compared to 1.02 in 2020. In the cage experiment, a local annual weed, *Bidens pilosa,* was associated with the highest number of aphid mummies (8.3) whereas the lowest number of mummies were observed in cages with *Tagetes minuta* (3.4). Through molecular identification, *Aphidius colemani* was found to be the most abundant parasitoid responsible for bean aphid control. During the off-season, results showed a positive significant correlation of natural enemy abundance and plant diversity (*P=0.002*). These results demonstrate the potential of plant-rich field margins to conserve and support natural enemy populations for natural pest regulation of aphids.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
BLAST	Basic Local Alignment Search Tool
BOLD	Barcode of Life Database
CABI	Centre for Agriculture and Bioscience International
COI	Cytochrome Oxidase I
DNA	Deoxyribonucleic Acid
HSD	Honestly Significant Difference
ICIPE	International Centre of Insect Physiology and Ecology
KARI	Kenya Agricultural Research Institute
KALRO	Kenya Agricultural and Livestock Research Organization
mtCOI	mitochondrial cytochrome *c* oxidase subunit I
NCBI	National Centre for Biotechnology Information
PCR	Polymerase Chain Reaction
RCBD	Randomized Complete Block Design

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Legumes are vital for food and nutrition security and serve as a source of income and livelihood for millions of people in Sub-Saharan Africa (Odendo *et al*., 2011). *Lablab purpureus* L., commonly known as dolichos or hyacinth bean has been categorised as ‘orphan legume crop’ and underutilised (Varshney *et al*., 2009) due to the little attention it has received in terms of global cultivation, utilisation and research (Khourya *et al*., 2014). Despite climate change, dolichos have a high capacity to enhance food security in Africa though its role in climate smart agricultural systems remains poorly understood. Dolichos serves various purposes, including food, livestock feed, and soil fertility management. It is valued for both its green and dried seeds for human consumption and as fodder for livestock. The crop's drought resilience makes it a valuable option for mitigating climate change risks to food supplies (Minde *et al*., 2021). However, dolichos production faces significant challenges, particularly from insect pest infestations. Major insect pests include pod borers *(Helicoverpa armigera* and *Maruca vitrata*) and the black bean aphid (*Aphis fabae*), with the latter causing severe economic losses. High aphid infestations can result in 20-40% yield loss, with some reports indicating up to 90% (Ahmed *et al*., 2004; Abate & Ampofo, 2003; Nahashon *et al*., 2016).

Synthetic insecticides have been widely used to manage these pests due to their fast action and broad-spectrum efficacy. The usage of these chemicals has limitations, including the suppression of both beneficial and harmful insects, reduction and killing of natural enemy diversity and subsequently reduced benefits of natural pest regulation (Stevenson *et al*., 2017; Yano, 2006; Foster *et al*., 2002; Harris & Dent, 2000). This side effects have increased the global push towards reducing the use of synthetic insecticides without compromising on agricultural production. This calls for an urgent need for finding environmentally friendly alternatives to replace the conventional agricultural practices (Bommarco *et al*., 2013).

Biological control is a crucial integrated pest management (IPM) tool offering a more environmentally benign alternative to synthetic pesticides (Kumar *et al*., 2018). It is the purposeful usage of living organisms which include parasitoids, predators and pathogens as pest control agents. This approach has proven to be of significant importance since most bio agents are naturally occurring. Nyaanga *et al*. (2008) reported that about 4200 aphidophagous coccinellid beetle species exist naturally.

Approaches to biological control include mass rearing of bio control agents in laboratories and releasing them into the field (inundative) or conservation which involves manipulating the environment to make it favourable for reproduction and survival thus enhancing their population (Ferron & Deguine, 2005; Ostman *et al*., 2003; Zehnder *et al*., 2007). Conservation biological control approach involves habitat manipulation by enhancing diversification of plant species in time and space in annual cropping systems (Bengtsson & Weibull, 2005; Bianchi *et al*., 2006; Rand *et al*., 2012). Increasing plant diversity within cropping systems offers an ideal environment for the natural enemies to survive and contribute towards the reduction of insect pests' infestation and crop damage. Landscapes with high plant species diversity have been reported to have abundance of natural enemies such as parasitic wasps and predatory beetles than in simplified landscapes with large monocultures (Chaplin-Kramer *et al*., 2011). This is because they offer structures that serve as permanent habitat for nesting, overwintering, and other food sources (Bianchi *et al*., 2006). Spillover, which is the term for the movement of species over the interface between crop and non-crop ecosystems, can amplify these landscape effects within crop fields. (Tscharntke *et al*., 2005). Many a times the effect of landscape on pest distribution is not well understood and it appears to vary within cropping systems and across the years.

Aphids are known to have very many natural enemies of different taxa. However, the efficiency of natural enemies in controlling aphids is often limited by lack of food, oviposition sites and refuge for their survival. Destruction of non-crop surroundings and the seasonal nature of field crops results in simplified landscapes of low plant diversity which negatively impacts on the operation of natural pest control (Van Emden, 1990). Little research attention has been done on integrated pest control, particularly the function of field margin plants in enhancing natural pest control in orphan legume crops such as dolichos. There is inadequate information and understanding on the role of field margin in conserving or enhancing the population of natural enemies and their subsequent potential for natural pest regulation. The understanding of how pest control services function and maintain a stable human-modified environment is limited, which hinders the implementation of ecological intensification in agriculture (Rusch *et al.*, 2013). The current study sought to investigate the roles of ecological intensification schemes in the conservation and increase of the diversity and abundance of natural enemies in different cropping systems.

1.2 Statement of the Problem

Aphids represent a significant insect pest species for dolichos beans (*Lablab purpureus* L.) causing yield losses of 20-40%. The damage is mainly caused through sucking of sap from plant tissues and transmission of viruses. Control of aphids is often difficult, due to their high reproduction and dispersal capacity. This has forced dolichos farmers to over-rely on synthetic insecticides as a control strategy. The use of synthetic insecticides has certainly contributed to significant yield gains. However, indiscriminate and injudicious application of synthetic insecticides in dolichos crop production has led to high costs of production and negatively impacted on the environment, human beings and beneficial organisms. Moreover, insecticides are only a temporary remedy with a constant demand for more application because insect pests often develop resistance and there is always continuous migration of more aphids into the farms. Therefore, exploring alternative aphid control methods, particularly through natural enemies, is crucial for integrated pest management (IPM) and is gaining attention as an eco-friendly approach with significant ecosystem benefits.

1.3 Objectives

1.3.1 Broad objective

To contribute to food and nutritional security of smallholder farmers through the use of field margin vegetation to conserve natural enemies for ecological management of aphids in dolichos.

1.3.2 Specific objectives

The study determined the;

i. Effect of field margin vegetation diversity and cropping systems on bean aphids and their natural enemies in dolichos.
ii. Effect of planted field margin vegetation species and cropping systems on bean aphid natural enemy populations and aphid damage in dolichos.
iii. Influence of floral resources abundance and diversity on conservation of natural enemies of bean aphids during periods outside the dolichos cropping season.

1.4 Hypotheses

i. Field margin vegetation diversity and cropping systems have no effects on bean aphids and their natural enemies in dolichos.
ii. Planted field margin vegetation species and cropping systems have no effects on bean aphid natural enemy population and aphid damage on dolichos.
iii. The abundance and diversity of floral resources which support natural enemies have no effect on the conservation of natural enemies of bean aphids during periods outside the dolichos cropping season.

1.5 Justification

Food security in Kenya and the world requires increased crop productivity to meet the demand for the growing population projected to be nearly 10 billion people by the year 2050 (Steensland & Thompson 2019). According to FAOSTAT (2017), Kenya's annual agricultural production needs to increase by approximately 75% in order to meet the food demand of a projected population of 70 million people by 2030. The continued agricultural intensification to escalate the production per unit area has resulted in natural habitat destruction and loss in biodiversity (Foley *et al*., 2011; Rockstrom *et al*., 2009; Schneider *et al*., 2015). Dolichos bean is a multipurpose legume crop that can be utilised as human food and animal feed though it remains underutilised. There is a need to prioritize promotion of this multi-purpose, drought and soil acidic tolerant legume in both commercial and smallholder farming systems to bridge the gap and achieve the Sustainable Development goal (SDG) 2 of zero hunger.

Aphids are major pests of dolichos bean production causing significant yield losses of up to 40%. The pest menace has been exacerbated by the monoculture farming practices and indiscriminate continuous application of synthetic insecticides. Increased concerns have been raised on the side effects of synthetic insecticides on non-target and beneficial insects, increased risks to human health and the environment. There is a need for more research focused on the development of ecologically friendly pest management strategies such as conservation biological control. Reports indicate that the low adoption of biological control-based systems is majorly due to poor understanding of the ecological complexity of farming systems (Nyaanga *et al*., 2008). Many farmers are not conversant with the potential and importance of natural enemies of bean aphids and are not able to estimate the control potential.

To achieve effective control of pests, natural enemies need to be identified and conserved around the farms throughout the cropping season. Natural enemies can be conserved and supported by increased plant species diversity within and around farms to provide refuge and non-prey food. The diversity of natural enemies of bean aphids on dolichos has not been determined in Kenya. Furthermore, natural enemies such as parasitoids are a highly diverse group of insects. For instance, the subfamily Aphidiinae (Hymenoptera; Braconidae) which is known to be parasitic on bean aphid contains over 400 species of aphid parasitoids. Many of these species have not yet been identified due to the presence of cryptic species that are challenging to differentiate morphologically (Smith *et al*., 2008). To give conclusive results, this study sought to identify parasitoids using DNA barcoding and predators of aphids on dolichos, their diversity within the field margin vegetation and their effectiveness in aphid control on dolichos and grain yield in Kenya.

CHAPTER TWO
LITERATURE REVIEW

2.1 Description, production and utilisation of dolichos bean

Dolichos (*Lablab purpureus* L.) (Family: Fabaceae) is multipurpose legume that is used as human food, animal feed and serves as a cover crop for soil conservation (Robotham & Chapman, 2017; Schultze-Kraft *et al*., 2018). It is native to Africa and cultivated throughout the tropics and thought to be the most ancient crop among cultivated plants (Ewansiha *et al*., 207). Wild dolichos species occur only in eastern and southern Africa and are considered the centre of origin, with Ethiopia as one of the potential centres of origin (Maass, 2016). It has many local names such as hyacinth bean, dolichos bean, seim bean, lablab bean, Egyptian kidney bean, Indian bean, chicharo, Australian pea and njahi in some parts of Kenya (Nord *et al*., 2020). Despite the fact that dolichos is a drought-tolerant crop grown throughout the tropical regions of Asia and Africa, it can as well be grown in a wide range of environmental conditions (Engle & Atoveris, 2000; Guretzki & Papenbrock, 2014).

Dolichos exhibits significant high species diversity, with subspecies identified based on growth habits, flowers, pods, and seeds (Maass *et al*., 2005; Pengelly & Maass, 2001). The plant can be semi-erect or climbing, with stems reaching up to 0.9 metres for erect cultivars and up to 7 metres for climbing types (Guretzki & Papenbrock, 2013; Mondal *et al*., 2017). Its extensive root system includes a vigorous taproot capable of accessing soil water up to 2 metres deep, contributing to its drought resilience (Grotelüschen *et al*., 2014; Kilonzi *et al*., 2017). The plant features long-stemmed trifoliate leaves, with leaflets varying from triangular to oval and measuring 7-15 cm in length, depending on the cultivar (Morris, 2009). Inflorescences consist of racemes with flowers in purple, blue, or white (Maass *et al*., 2010). Pods are 4-10 cm long, smooth, flat, and pointed, containing 2-5 seeds that may be white, cream, pale brown, dark brown, black, or mottled (Mondal *et al*., 2017; Reddy *et al*., 2018).

Production of dolichos in Africa is spread out in small regions of East Africa (Uganda, Tanzania and Kenya) and West Africa (Cameroon and Nigeria) (Chawe, 2019; Forsythe, 2019; Pauline, 2020). However, the production and demand for dolichos in these regions have decreased over the years (Nedumaran *et al*., 2015). This reduction has been evidenced when farmers reported approximately 10% reduction in land used for dolichos production (Abate *et al*., 2011; Raghu *et al*., 2018). Most dolichos production in Kenya is for domestic consumption with imports from Tanzania to meet demand (Boit *et al*., 2018; Nahashon *et al*., 2016). However, the exact amount of dolichos being produced in Kenya and Tanzania is unknown due to informal, undocumented trade and is estimated to be only 20, 000 ha with 0.4 tons/ha of grains with low domestic demand (Forsythe, 2019; Nedumaran *et al*., 2015). The market survey revealed that the low demand is due to changing eating habits, long cooking times of grains and poor palatability of the common black seeded variety (Kilonzi *et al*., 2017; Ngure *et al*., 2021).

Dolichos possesses high nutritional value but remains underutilised globally due to limited cultivation and usage (Maass *et al*., 2010; Varshney *et al*., 2009). It can be grown either as a sole crop or intercropped with maize (*Zea mays*), finger millet (*Eleusine indica*), or sorghum (*Sorghum bicolor*) (Cheruiyot *et al*., 2011; Stefani *et al*., 2015). The pods are rich in carbohydrates, proteins, fats, fibre, and essential minerals such as calcium, phosphorus, and iron (Kilonzi *et al*., 2017). Protein content in the pods and seeds ranges from 10-19% and 15-25%, respectively (Naeem *et al*., 2009). Dolichos protein isolates can improve food products like cakes by enhancing volume, texture, colour, and shelf life (Subagio & Morita, 2008). The bushy varieties of Dolichos can be conserved as hay or fed green to dairy cattle (Guretzki & Papenbrock, 2014; Maundu *et al*., 1999; Maass *et al*., 2010). Additionally, using Dolichos as green manure has been shown to enhance soil nitrogen availability (Cheruiyot *et al*., 2011; Northup & Rao, 2015).

In Kenya the production scale of dolichos beans is not well documented, despite its long history in the country. The Rongai cultivar, one of oldest known varieties, is believed to have originated from the Rongai area (Cameron, 1988). This bean is often intercropped with maize, finger millet, groundnut, sorghum or grown as a monocrop. It acts as a cover crop with dense green foliage that helps protect the soil and conserve moisture by reducing wind and rain erosion (Mureithi *et al*., 2003). Dolichos beans hold significant potential as a valuable resource for tropical agricultural systems, contributing to both human nutrition and animal feed (Pengelly & Lisson, 2002).

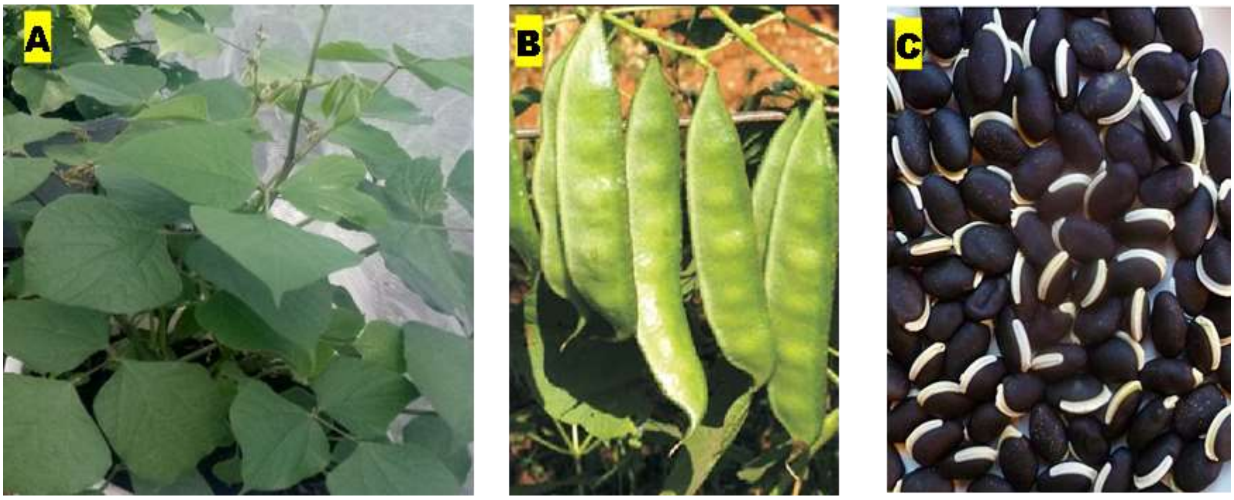

Plate 2. 1: Dolichos crop at vegetative stage (A), podding (B) and grains(C).

2.2 Major insect pests of Dolichos

Insect pests are a major biotic factor that significantly reduces Dolichos yields, accounting for up to 50% of total losses (Boit *et al*., 2018; Forsythe, 2019; Mondal *et al*., 2017; Pervez & Chandra, 2018). The severity and incidence of pest attacks vary based on location, varieties used, environmental conditions, and agronomic practices (Mwanauta *et al*., 2015). Research has identified 55 species of insects and mites that feed on Dolichos from the seedling stage to harvest. Major pests include sucking pests such as aphids (*Aphis craccivo*ra and *Aphis fabae*), lablab bug (*Coptosoma cribraria* and *Riptortus pedestris*), as well as a complex of pod borers including *Maruca testulalis*, *Helicoverpa armigera*, *Adisura atkinsoni*, *Etiella zinckenella*, *Cydia ptychora*, *Exelastis atomosa*, and *Lampides boeticus* (Ahmed *et al*., 2004; Maradi *et al*., 2019).

Aphids (Hemiptera: Aphididae) are particularly significant pests for dolichos and other legumes such as soybean (*Glycine max*), pigeon pea (*Cajanus cajan*), and common bean (*Phaseolus vulgaris*) (Mwanauta *et al*., 2015; Pervez & Chandra, 2018; Reddy *et al*., 2017). They reproduce rapidly, leading to high population densities that cause economic yield losses (Mondal *et al*., 2017). Aphid species vary with the host plant with the black bean aphid (*Aphis fabae*) being the most prevalent species attacking dolichos (Bachmann *et al*., 2014; Boit *et al*., 2018; Mondal *et al*., 2017; Singh & Singh, 2017).

2.2.1 Taxonomy and biology of bean aphids

Aphids belonging to the order Hemiptera and family Aphididae encompass over 5000 species (Faria *et al*., 2016). They are small, soft-bodied insects with sucking mouth parts that mainly feed on phloem (Foottit *et al*., 2008). Aphids are polyphagous, inhabiting tropical, subtropical, and temperate regions globally, and infesting legumes, cereals, and vegetables (Valenzuela & Hoffmann, 2015). They are known to feed and colonise over 3000 species of herbaceous plants and shrubs (Blackman & Eastop, 2007). Bean aphids are capable of reproducing sexually and asexually rapidly increasing their population in what is sometimes referred to as "aphid explosions” (Blackman & Eastop, 2000). They are found in large colonies, adult aphids are about 2mm long, with a small head and bulbous abdomen (Abate & Ampofo, 2003; Mondal *et al*., 2017). Their antennae are less than two-thirds their body length, and their legs are pale yellow with black tips (Dixon, 2019). They have a pair of slender, elongated tubes near the rear of the abdomen, called cornicle, which produce a defensive waxy secretion (Kindlmann & Dixon, 2010).

Aphids are economically invasive pests throughout the world and are known to be the most destructive insect pests (Dedryver *et al*., 2010). They feed on sap from the plant phloem, or sieve elements. They have piercing and sucking mouthparts. Affected crop leaves turn yellow, wilt or fall off. Flower buds or fruit become distorted and malformed due to toxic saliva injected from the aphids (Dong *et al*., 2011). The direct damage caused on plants during sap sucking sap damage crop products and produce making them unsuitable for consumption (Dedryver *et al*., 2010; Ragsdale, 2007). In addition, aphids are trans-mitters of more than 200 plant viruses (Brault *et al*., 2010; Hogenhout *et al*., 2008) hence transferring plant viruses and their related diseases. In dolichos they transmit bean mosaic virus (Black man & Eastop, 2000).

2.2.2 Bean aphid life cycle and plant damage

The life cycle of the bean aphid includes several asexual generations interspersed with a single sexual generation (Buruchara *et al*., 2010). In the warm tropical regions of Africa, reproduction occurs only through parthenogenesis (Abate & Ampofo, 2003). During asexual reproduction, aphids can produce diverse phenotypes, including both winged and wingless forms (Petermann *et al*., 2010; Weisser, 2019). Wingless forms are prevalent when food is abundant and environmental conditions are favourable. Conversely, winged forms emerge when food is scarce or the colony becomes overcrowded (Mondal *et al*., 2017). These winged aphids migrate to establish new colonies and may invade bean fields shortly after seed emergence. (Singh & Singh, 2017). The lifespan of a parthenogenetic female is approximately 50 days, during which she can reproduce 40 to 60 offspring as shown in the life cycle (Plate 2.2).

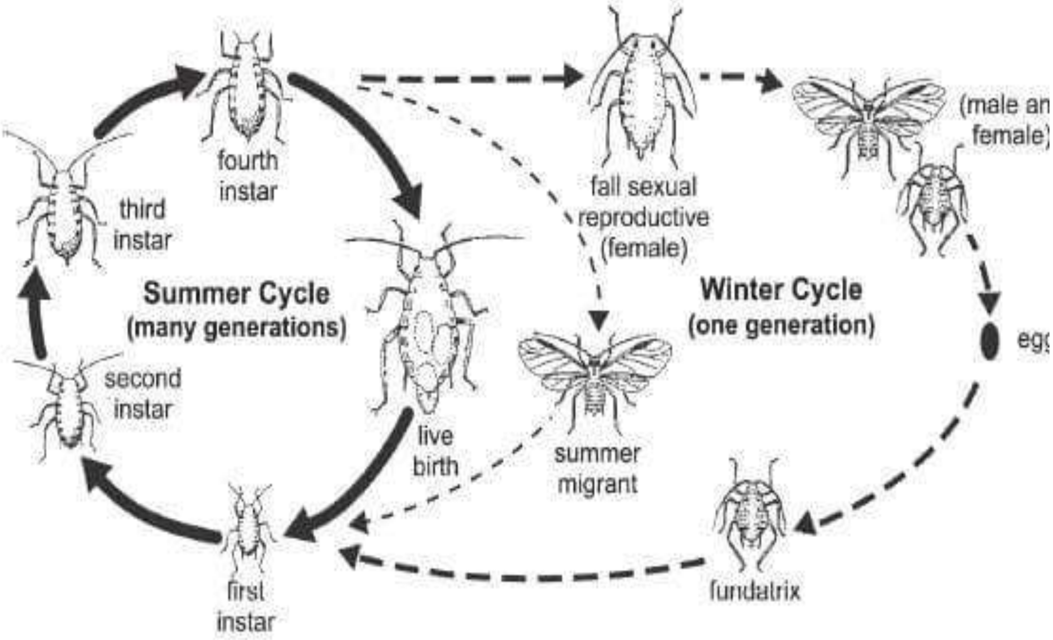

Plate 2. 2: Asexual and sexual reproduction life cycle of aphids

Source: Kang *et al*. (2008).

Ordinarily, in a few week hundreds to thousands of aphids on a plant are produced by less than a dozen aphid "colonizers" when the procedure is repeated multiple times, leading to huge population surges. The population of aphids usually build up till conditions are so congested or the plant losses turgidity after being fed before they develop wings and fly off to find new host (Mwangi *et al*., 2008). At any given time, these colonies suggest that the aphids have taken root on the plants and will soon start to multiply in numbers (Plate 2.3).

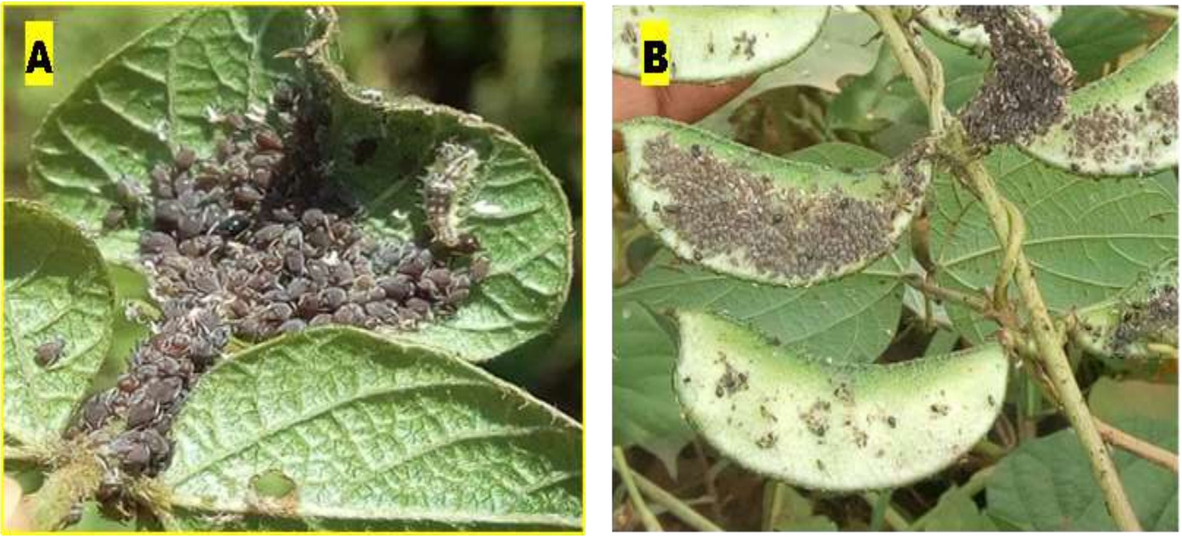

Plate 2. 3: Bean aphid colonies on dolichos leaves (A) and pods (B).

Aphid populations survive during off-season on alternate host plants such as weeds along roadside vegetation and farm boundaries like *Bidens pilosa*. During the cropping season, winged adults keep moving around farmlands to find a suitable host and young succulent crop. Aphid infestation normally starts from crop hedges close to alternative host vegetation around the crop fields (Simon *et al*., 2002). When the aphids land on a right host the adults lay some wingless young ones called nymphs on tender tissue of the plant (growing points) prior to moving out to find another new plant. The nymphs left behind quickly start feeding on plant sap and steadily rise in size, and in seven to ten days they become mature adults ready to reproduce live young ones (Wosula *et al*., 2017. Aphid infestation within the crop fields often occurs in patches or hotspots. Aphid colonies are found around the stems, growing points such as leaf and flower buds, flower clusters and the underside of leaves. Infested plants exhibit wrinkled leaves, stunted growth and deformed pods. Severe infestations can lead to plant desiccation and potentially death. Additionally, the honeydew excreted by the aphids contaminates plant parts, promoting the growth of black saprophytic moulds that reduce the photosynthetic area (Longley & Jepson, 1996).

Rainfall has a significant impact on the timing and quantity of aphid flights into crops; heavy rainfall encourages the establishment of appropriate host plants and permits aphid populations to increase early in the growing season. Aphid development rates slowdown in the cooler months. Warmer humid months trigger a sharp rise in aphid development rates, where reproduction and population development occur very rapidly (Brisson & Stern, 2006). Aphid pest explosion or rapid multiplication is very difficult to control in the farms because of the dynamic changes in the weather which leads to warm conditions favouring high reproduction rates. When this is coupled with the short life cycle and high numbers of nymphs reproduced from one adult it urgently demands that a farmer has to have a reliable control mechanism in place to manage the aphids. This pressing need has driven most farmers to injudiciously spray crops several times whenever there is any infestation, resulting in high pesticide use in farms. This management practice may not stop soon because it presents a quick fix for control of aphids. Consequently, it is important to develop alternate eco- friendly techniques of management to reduce the harmful effects of pesticides overuse. This can be through integration of field margin vegetation and use of natural enemies for eco-friendly regulation of aphid populations throughout the season.

2.3 Control methods of bean aphids

Bean aphids are challenging pests to manage using a single control strategy (Mwanauta *et al*., 2015). Their populations tend to increase during dry weather, making infestations more severe during dry periods (Wosula *et al*., 2017). Unfortunately, pest control efforts on orphan legumes, such as dolichos, often rely solely on chemical pesticides, which can harm farmers, consumers, non-target organisms, and the environment (Cork *et al*., 2009; Stevenson *et al*., 2017). The high reproductive rate of bean aphids complicates control efforts when using just one method (Mwanauta *et al*., 2015; Tembo *et al*., 2018). Therefore, it is essential to develop and implement integrated pest management (IPM) strategies that are safe and environmentally friendly. Farmers currently use various methods to manage legume pests, including cultural practices, chemical insecticides, and biological methods (Karani, 2017).

Integrated pest management combines various pest management strategies to maintain the pest population below the economic injury level (Roubos *et al*., 2014). Conservation One of the objectives of IPM is conserving natural enemies of pests by modifying the environment to protect these beneficial organisms and inhibit pest build up in fields (Nyaanga, 2008; Omkar & Pervez, 2016; Paredes *et al*., 2013). Providing suitable habitats with flowering plants in and around crop fields can support beneficial insects, enhancing their numbers and diversity for natural pest regulation (Paredes *et al*., 2013; Ramsden *et al*., 2014).

2.3.1 Cultural control

Cultural practices are among the oldest documented methods for pest management (Kebede *et al*., 2019). Despite their effectiveness, the use of cultural practices has declined as farmers increasingly rely on synthetic pesticides, leading to reduced research in this area (Abate & Ampofo, 2003). Effective cultural control requires detailed knowledge of the pests' biology, natural controls, and environmental interactions (Wosula *et al*., 2017). These methods aim to make the environment less attractive and less hospitable to pests while promoting natural pest control with the aim of reducing pest numbers below economic injury levels or to a point where biological controls can be effective (Shehu *et al*., 2001).

Smallholder farmers practice intercropping, growing beans alongside cereals like finger millet (*Eleusine indica*), sorghum (*Sorghum bicolor*) and maize (*Zea mays*) (Grotelüschen *et al.,* 2014). These practices are not effective alone as a control but are effective when in combination as several practices (Kebede *et al*., 2019). Abate and Ampofo, (2003) found that bean fly (*Ophiomyia* spp) adults emerged only in the presence of beans or other host plants like cowpea seedlings, but not in the presence of non-host plants like maize. Laboratory trials showed that bean fly larvae survived on bean and cowpea roots but not on soybean and maize roots. Cultural practices are safe and cost-effective hence their integration into insect pest management programmes will reduce reliance on chemical insecticides (Mwanauta *et al*., 2015).

2.3.2 Chemical control

Over the past 60 years, the use of insecticides has significantly improved food production (Sola *et al*., 2014). Synthetic insecticides are fast-acting and can kill a wide range of pests, but they also pose risks to beneficial insects and non-target organisms (Stevenson *et al*., 2017). In rural areas, insecticides are often limited in availability, sometimes adulterated, or improperly applied (Karani *et al*., 2017). While insecticides such as cypermethrin, carbamates, and pyrethroids are effective against pests (Maksymiv, 2015; Ndakidemi *et al*., 2016), there is a growing need for safer alternatives. Selective synthetic insecticides like indoxacarb and spinosad have been developed, which are less harmful to beneficial insects such as natural enemies and pollinators while still effectively controlling pests (Fernanda *et al*., 2017; Ulrichs *et al*., 2001). For instance, Mwanauta *et al*. (2015) reported that applying imidacloprid as a foliar spray at the podding stage of common beans (*Phaseolus vulgaris*) reduced populations of leaf miner Liriomyza huidobrensis (Blanchard) and its parasitoids.

Despite their effectiveness, synthetic pyrethroids have been widely used for controlling field crop pests (Bibi *et al*., 2014), but they can severely impact human health, natural enemies, and pollinators (Mwanauta *et al*., 2015). Increasing food safety concerns and environmental conservation efforts have driven the demand for organically produced food (Chaudhary *et al*., 2017). Consequently, there is a need for safe alternatives that pose low health risks and help conserve agricultural biodiversity, such as botanical insecticides and natural pest regulation methods.

2.3.3 Biological control

Biological control is considered as ecofriendly and effective means of reducing or mitigating pests and the damage they cause. It is the usage of living organisms to regulate the density of population of other living organisms (Van Lenteren, 2006). The difference between biological control and the use of synthetic pesticides is that natural enemies are often self-perpetuating and self-dispersing. As a result, biological control is regularly irreversible, although this is not always the case in inundative types of biological control. All ecosystems, both natural or man- made have natural control in place. This implies that all cropping systems have some degree of biological control in place. Parasitoids and predators are major biotic factor that can be used to suppress insect population in agricultural ecosystems (Symondson *et al.,* 2002). Numerous integrated pest management programs have been generated to support pest-resistant varieties, biological control and mating disruption aimed at minimising the use of synthetic insecticides (Prokopy, 2003).

In general, there are three distinct categories of biological control techniques used in pest management programs. These are; importation or classical biological control agents, augmentation and conservation. Classical biological control involves the deliberate introduction of an exotic (nonnative), typically co-evolved biological control agent for long-term and permanent establishment (Van Driesche, 2008). This is typically carried out by government agencies in complicated situations, such as when a pest's natural enemies are insufficient, which can occur when a pest is unintentionally introduced into a new region without its natural enemies. The second is inoculative release of relatively few natural enemies at a critical time of the season or inundative release of millions of natural enemies to a place. The release of exotic natural enemy species causes negative effects on non-target species and the ecosystems in which these species function; however, these effects might be prevented. These negative effects are not only determined on the target species, but also on indigenous non-target species. Otherwise, to reduce the risks associated with the release of exotic natural enemies would be to limit the number of releases by increasing the use of native natural enemies. The third is conservation of existing natural enemies in an environment.

Conservation biological control entails environmental manipulation to increase survival, physiological and behavioural performance for the natural enemies leading to an enhanced efficiency (Grez *et al*., 2014; Kati *et al*., 2021; Knapp & Řezáč, 2015). Conservation of natural enemies demonstrates its potential in pest management scenarios. This is done to natural enemies that are already used to the surroundings and to the target pest (Lavigne *et al*., 2018). These natural enemies tend to have three attributes in common. These attributes are; colonising ability to allow the enemy to keep pace with the spatial and temporal distribution of the habitat. Temporal persistence in the absence of the target pest. Finally, opportunistic feeding habits allow for persistence and permit the natural enemy to rapidly exploit the pest population. Conservation can be accomplished easily and economically with the modification of vegetation. The application of classical biological management can effectively manage primary and secondary pest populations while mitigating the risk of pest outbreaks and resurgences (Johnson *et al*., 2008).

2.3.4 Natural enemies in control of aphids

Natural enemies, especially parasitoids and predators, are the major groups in the natural pest regulation. Integrated Pest Management (IPM) is considered a more sustainable approach for pest control since it relies on the most economical means of control with the least hazard exposure to humans and the environment. Pest control by natural enemies is an essential ecosystem service valued at $4.49 billion annually in the USA alone. Natural pest regulation represents an important ecosystem service for agriculture (Losey & Vaughan, 2006), with a major hope in sustainable pest management for the future (Bommarco *et al*., 2013). Increased pest pressure, without effective biological control or pesticide alternatives, can facilitate pest evolutions resistance to various classes of insecticides and biologicals. It demands that within the context of increasing global pressure to increase crop yields to feed the growing human population, precautions are taken to reduce use of harmful effects of agrichemical inputs and practices. This can be done by adopting agricultural practices that do not negatively impact the environment such as the use of natural pest control.

Aphids have been identified as significant agricultural pests across the world, and a number of natural enemy groups are known to offer some degree of control over aphid infestations (Schmidt *et al*., 2003). Use of natural enemies in the management of aphids is a very promising option, estimated to account for at least 50% of pest control measures (Pimentel, 2005). There are numerous groups of natural enemies that are known to provide biological control of aphid consisting of predators and parasitoids (Schmidt *et al*., 2003). Parasitoids like parasitic wasps are able to locate aphid colonies from a long distance by “alarm signals” emitted via an infected/ fed on plant. After locating the aphid, the wasps use their ovipositor to lay eggs (oviposit) into the aphid abdomen. The eggs are laid on the back side of the aphid abdomen through a piercing ovipositor which leaves a small, barely visible reddish mark. Inside the abdomen egg/eggs hatch into larva/ larvae and start their developmental cycle. In the initial days the larva feeds from within without affecting the aphid activities but as it increases in size it damages more aphid tissues and the aphid becomes swollen, papery, and a silvery brown in colour, turning them into hard, aphid-shaped shells called mummies (Schmidt *et al*., 2003). As the larval instar stages increase towards adult it consumes the pest abdomen completely from inside and cuts a small hole, called an exit hole where it emerges from as a flying adult to seek new prey as shown below (Plate 2.4). Parasitic wasps are found in the families; Aphelinidae, Braconidae and Encrytidae (Tomanovic *et al*., 2003).

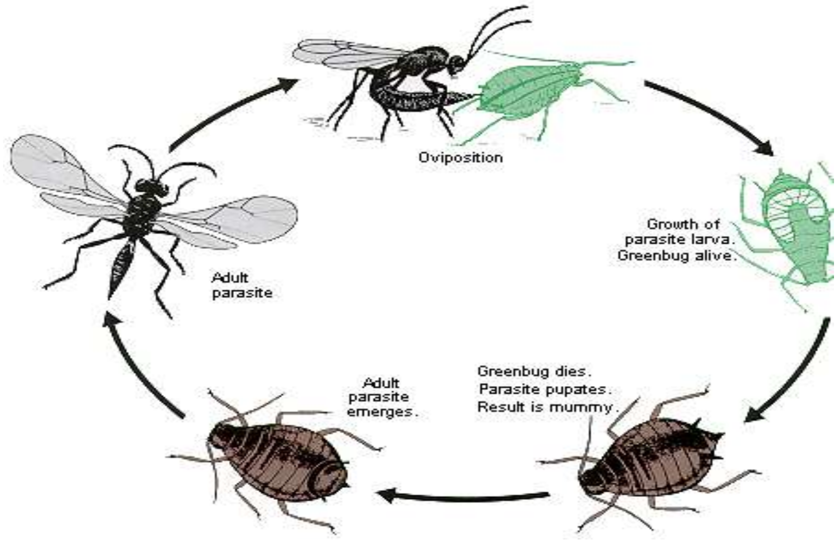

Plate 2. 4: Life cycle of aphid parasitoids.

Source: htttp: //soilcropandmore.info/crops/legume/Insects/b-5044-3.gif

Parasitoids are a highly diverse group of insects. The subfamily Aphidiinae (Hymenoptera; Braconidae) alone contains over 400 species of aphid parasitoids (Derocles *et al*., 2016). Many parasitoid species are yet to be identified (Hebert *et al*., 2016), in part due to the prevalence of these cryptic species that are difficult to distinguish morphologically (Smith *et al*., 2008). *Aphidius spp.* are economically and ecologically important parasitoids regulating aphids naturally and promoted as commercial biological control agents (Fernandez & Nentwig, 1997) (Plate 2.5). Therefore, they are a key group to study to better understand their diversity, biology and population structure in relation to pest control. Many *Aphidius* spp (Petermann *et al*., 2010; Prado *et al*., 2015) have relatively low intraspecific genetic variation and in some cases also low interspecific diversity (Kos *et al*., 2011; Tomanović *et al*., 2014). For example, *Aphidius uzbekistanicus* Luhetzki and *Aphidius avenaphis* Fitch show very little genetic variation in the cytochrome oxidase subunit I (COI) barcoding region, with genetic distance in this group of up to 0.2% (Tomanović *et al*., 2013).

Other generalist natural enemies are majorly predators that prey on aphids. They include carabid, staphylinid beetles and spiders, which mainly colonise pests from the ground (Schmidt *et al*., 2003). The other important aphid predators include: lady beetles of several species, lacewings, big eyed damsel and minute pirate bugs. Predators directly consume or feed or prey on one aphid species or several species (Desneux & Ramirez-Romero, 2009; Dixon, 2000). In addition, birds represent the top predators for insects in many agricultural systems.

The ladybird beetles are common biological control agents of aphids in natural field settings (Agarwala *et al*., 2017; Ahmad *et al*., 2017). They delay and prevent aphid outbreaks and densities (Heimpel & Asplen, 2011). They have the ability to regulate aphid population due to the aspects of being voracious with good searching ability, high predation capacity as adults and larvae stages and high reproduction rates (Amorós-Jiménez *et al*., 2012; Weisser, 2019) (Plate 2.5).

The carabids are typically polyphagous; however, they are voracious feeders, consuming prey equivalent to their own body mass daily. They have specialised feeding habits and feed mostly on bean aphids that fall to the ground floor. The carabidae larvae are more carnivorous and restricted in food range than adults. Results from Kromp (1999) showed that gut dissections and ELISA tests identified 21 species of adult carabids to have fed on aphids. Aphid feeding occurs mainly early in the season when aphid density is still low.

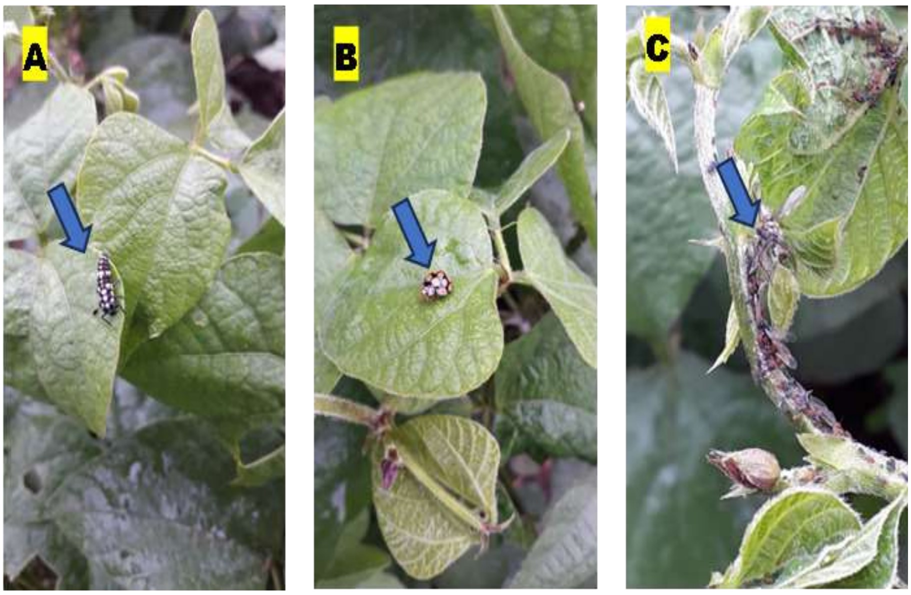

Plate 2. 5: Bean aphid natural enemies; ladybird larvae (A), ladybird adult (B) and aphidius parasitoid (C).

2.3.5 General ecology and distribution of natural enemies

Natural enemies/ beneficial living organisms which reduce pests and diseases are usually present in any crop unless broad spectrum pesticides have been used to kill them. Areas which have not been cultivated or disturbed contain hundreds and thousands of species which tend to form a balance with each other due to dependency relationship. Although occasional outbreaks may occur, any particular species is not likely to build up a large population if the organism that feeds on it (natural enemy) is also present (Tylianakis & Romo, 2010). Natural enemy numbers vary over time because many populations follow a similar pattern of fluctuation as their hosts or prey because of inter- dependency. Many natural enemies multiply in response to the availability of the food, hence there is a "time lag" between pests and natural enemy fluctuations and buildups. Natural enemies are highly mobile and their movement in and out of the crop will depend on how far their refuge places are and availability of prey and food. In a normal trend, all season round there will be fluctuations in natural enemy population, hence the need to assess build up at the different crop stages (Zhao *et al.*, 2013). It is worth noting that as the plant growth physiology changes so do the associated organism species and population. Likewise, as the crop and margin species change so do the natural enemies and pests and this influences their succession and colonisation (Chaplin-Kramer *et al.*, 2011).

Aphids, cicadellids, thrips and flies are some of the pests that are most effective in colonisation of crop niches and are numbered among the worst pests. In contrast their predators and parasitoids are slower in colonising vacant crop sites. Normally there is no selective pressure for the natural enemies to colonise early because they will experience food shortage. The difference in colonising ability of the natural enemies coupled with difference in reproduction rates when not equitable to the pest leads to early pest outbreaks during the crop season before natural enemy populations reach the climax (Bianchi *et al.*, 2007).

The survival of herbivorous insects is affected by various factors such as; quality and quantity of host plants, attack by predators, parasitoids and infection by disease within an ecosystem. Evaluating the effects of these multiple factors together is essential in providing a better understanding of the interactions among multiple trophic levels. In landscapes with high numbers of complex or semi natural habitats, natural enemies such as parasitoid wasps, predatory beetles, and insectivorous birds are frequently more abundant than in simplified landscapes of monoculture plants (Bianchi *et al.*, 2006). These effects vary depending on the natural enemies being considered in the spatial scale of observations (Chaplin-Kramer *et al.*, 2011). Similarly, in the same study it was observed that effective natural enemies are more abundant in permanent habitats. These areas act as conservation places where natural enemies build structures for nesting, overwintering and access food resources (Bianchi *et al.*, 2006). Increased plant species' diversity in the landscape enhances or encourages spillover of organisms occurring within the crop and non-crop habitats (Tscharntke *et al.*, 2005). In contrast, effects of landscape context on pest distributions are less well understood and appear highly variable between systems and years (Bianchi *et al.*, 2006; Chaplin-Kramer *et al.*, 2011). Pest population distributions are a reflection of landscape effects on the pests themselves and effect of their suppression by natural enemies in the same landscape over more than one season (Chaplin-Kramer & Kremen, 2012).

Landscape diversification is a key element for focused development in sustainable agriculture. It is an ecologically based approach which aims at providing favourable habitats for natural enemies to stabilise the biological control in an agro-ecosystem. It provides favourable conditions for feeding and reproduction of the natural enemies (Landis *et al*., 2000). Landscape diversity provides natural enemies with resources such as favourable microclimate, alternative host prey or plant-based food such as pollen, nectar or honeydew (Heimpel & Jervis, 2005). Most plant species display various features to attract pollinators such as flower colour shape and odour (Nocentini *et al*., 2012; Schiestl & Johnson, 2013). Hence, conservation of non-crop habitats around the crop fields such field margin vegetation forms part of habitat management that promotes a better option for a sustainable pest management system (Rand *et al*., 2012).

Agricultural intensification has resulted in a simplification of landscapes by the expansion of agricultural land, enlargement of field sizes and removal of non-crop habitats which has affected the functioning of natural pest regulation. This is due to the fact that non-crop habitats provide food for a broad spectrum of the natural enemies and provide refuge for natural enemies between crop and non-crop habitats. The expansion, up-scaling of field sizes and clearing of agricultural land have resulted in a simplification of agricultural landscapes containing only small fragments of natural habitats. These changes in land use, in combination with high agrochemical input in crop fields, are the primary causes for the rapid decrease of biodiversity and the occurrence of invertebrate taxa in many of these landscapes (Benton *et al*., 2003; Robinson & Sutherland, 2002).

Most available literature have a consensus that it is possible to control insect pests using natural enemies but only differ in principle of their effectiveness. According to Rodriguez and Hawkins (2000) and Finke and Denno (2004), a simplified natural enemy community provides control of pest populations that is equal to or better than a complex of natural enemies. Martin *et al*. (2015) documented that non-crop habitats such as field margins, fallows, hedgerows and wood lots are relatively undisturbed. They offer or provide temporary habitats for natural enemies within the landscape which is an important factor in insect pest suppression approach in the farms. Results from Bianchi *et al*. (2007), states that small-scale landscapes that are rich in non-crop habitats with extended crop and non-crop interfaces allow effective early season field colonisation by natural enemies. Tylianakis and Romo (2010), reported that many nectar-feeding natural enemies such as parasitoids, syrphids and chrysopids are more abundant near field edges than in field centres and in small-scale landscapes with extended crop and non-crop boundaries. In addition, a relatively large proportion of the crop area is subject to increased activity of natural enemies. Sahrawat *et al*. (2018), confirms that; natural pest regulation in agriculture is provided by wild invertebrates which are dependent on flowering plants in field margins which provide food as nectar or pollen or alternative insect hosts.

The suppression of pest populations in crops by natural enemies as opposed to synthetic pesticides provide eco-friendly control strategies that can reduce yield loss without the harmful effects to the environment (Ostman *et al*., 2003). There is mixed information and understanding of the role of plant species biodiversity in maintaining natural pest control. Hence, there is a need to conduct more studies to provide information in some of the knowledge gaps.

2.3.6 Cropping systems and their influence on natural enemies and biological control

Farming systems and crop management with greater reliance on ecosystem services such as biological control of insects are thought to increase the sustainability of agroecosystems (Li *et al*., 2021; Mansion-Vaquié *et al*., 2020). They have been shown to have major effects on species composition, abundance, and distribution in agroecosystems (Rayl *et al*., 2018). Modern systems such as monoculture are ecologically unstable and may cause serious pest problems such as outbreaks and major pest niche dominance (Sánchez-Bayo, 2021). Instability of agro-ecosystems can be caused by the vegetational simplification, resulting from the adoption of vast crop monocultures (Zhang *et al*., 2021). These systems are economically beneficial since they promote large scale productions, operations are mechanised reducing on production costs translating to high yields and profit margins. This new agricultural adopted methods of using advanced equipment and ways of production satisfies the farmers needs in a reliable and timely manner. However, in the long run it tends to increase pests and pesticide use since they promote niches and favourite food resources specific to organisms/ pests for extended periods or year-round. As a result, they reproduce faster and more efficiently and maintain the high populations throughout the season if not controlled. Over time, as farmers struggle to attain high yields and control such populations, especially by use of various classes of pesticide they can develop pesticide resistance. Additionally, the pesticides reduce organisms' abundance by directly increasing the mortality or reducing the fecundity of the target species (Sánchez-Bayo, 2021).

Increasing plant diversity within cropping systems is an important phenomenon that offers an ideal environment for the natural enemies to survive in agro ecosystems and contribute to the reduction of insect pest' infestation and damage on crops (Munyuli *et al*., 2007). This is a perspective of pest control that involves habitat manipulation by enhancing diversification of plant species in time and space in annual cropping systems (Bengtsson &

Weibull, 2005; Bianchi *et al*., 2006; Rand *et al*., 2012). The densities of natural enemies tend to be greater in polycultures than in monocultures and promotion of biodiversity in agro-ecosystems regularly favours natural enemies, suppresses pests and, in some cases, reduces crop damage.

2.3.7 Field margin vegetation in supporting natural enemies

Natural pest regulation by use of field margin vegetation (FMV) is considered one of the most important services of biodiversity with an estimated value of more than 400 billion dollars (US) per year worldwide (Rusch *et al*., 2010). Community structure, species richness and abundance, and population dynamics and interactions within and between trophic levels are affected by spatial context. FMV influences natural enemy communities through the provision of food and shelter resources. These resources may be foraging patches, breeding sites, or overwintering sites. Landscapes with large amounts of semi-natural habitat may benefit natural enemies by providing undisturbed areas that offer shelter from crop disturbances, overwintering refuges, alternative hosts and prey, and additional nectar resources. This in turn has previously been related to high populations of predators and parasitoids thus greater pest reduction on field crops. Studies by Woltz *et al.* (2012) showed that Coccinellid abundance was higher in buckwheat than in control field margins in all landscapes, and coccinellid abundance in soybean was positively related to the amount of semi-natural vegetation in the landscape. FMV can also be manipulated by planting floral source strips leading to increased predator or parasitoid abundance and reduced pest pressure in crop fields. However, the distance between fields and semi natural areas and their spatial organisation are therefore important in determining insect population dynamics.

Conservation biological control is considered to be the cornerstone of IPM (Nyaanga, 2008) where predators and parasitoids are provided with a suitable environment for survival.The success of conservation biological control can be enhanced by reducing the indiscriminate and excessive use of synthetic pesticides that pose both direct and indirect toxic effects on natural enemies (Khan *et al*., 2015). The population of natural enemies is difficult to conserve with the application of pesticides because of their sensitivity to insecticides as compared to the insect pests (Pezzini & Koch, 2015). Field study by Ochieng *et al*. (2022) demonstrated the potential field margin vegetation in reducing bean aphid populations and higher recovery of natural enemies after application of insecticides. In addition, in the presence of field margin vegetation a higher grain yield and above-ground biomass were observed which was partly attributed to natural pest regulation of bean aphids.

Field margins have been reported to have effects on natural enemies' diversity and density of insect pests. Natural enemies often need a greater diversity of resources other than the crops for additional prey, floral resources, or suitable reproduction sites (Ramsden *et al*., 2014; Sorribas *et al*., 2016). An increase in the abundance of natural enemies is thought to augment pest control through parasitism and predation at varying levels (Bianchi *et al*., 2006, 2008; Thies *et al*., 2008). Planted field margin plants have been proven to be a promising technology for conservation biological control (Mkenda *et al*., 2019). Plant species such as blackjack (*Bidens pilosa*) have been shown to support a higher abundance of coccinellids (Quispe *et al*., 2017). Flowering plants are known to have extrafloral nectaries that attract natural enemies to supplement their diet (Ben-Issa *et al*., 2017). Adults of beneficial insects including predators and parasitoids require nectar to complete their diet and provide energy requirements enhancing their prey search (Quispe *et al*., 2017). In addition, extra floral nectaries have been shown to induce higher longevity, fecundity and predation rates of natural enemies (Charles & Paine, 2016; Lundgren, 2009). Wäckers and Van-Rijn (2012) reported that in the absence of prey, nectar can strongly support survival as well as locomotion of coccinellids. Pan *et al*. (2020) indicated that aphidophagous ladybird beetle adults and larvae feed on aphids as a large part of their diets, although they may occasionally use alternative floral resources such as plant pollen and nectar at low aphid density. The study further indicated that adult ladybird beetles usually disperse to these non-crop habitats to lay eggs.

In some systems, there is a positive correlation with pest densities indicating that pest densities may, at times, drive parasitoids (Costamagna *et al*., 2004; Thies *et al*., 2005). According to Chaplin-Kramer and Kremen (2012) local diversification, from within field or around field hedgerow sources, can improve natural pest control. Therefore, it is important to evaluate on-farm diversification approaches may lead in suppression of insect pests.

2.4 Classification of aphids and their natural enemies

It is important to have a reliable species identification method for monitoring population dynamics of insect pests in space and time. Additionally, to enable rapid detection of invasive potential insect pests and disease-causing pathogens at farm level. Different methods have been used to classify insect pests including the aphids and their natural enemies. The methods include the old conventional classification method that relies on morphological dichotomous classification keys and the current DNA barcoding methods.

2.4.1 Conventional classification of insects

Conventional classification is done based on the overall similarities; across insect species is mostly based on visible characteristics showing on their bodies. It is based on description of the physical form of the insects as seen by a taxonomist and classified with the aid of dichotomous taxonomic keys (Shaw & Huddleston, 1991). Generally, aphids and their natural enemies have been identified using morphological characters. However, their small size, polymorphism, environmentally induced variations and reduction in morphological characters can make identification very difficult (Miller & Foottit, 2009). Likewise, the routine morphological identification of insects is time-consuming and requires specific knowledge, training and skills that may take years to acquire. The accurate identification of many insects is difficult, because many species are morphologically similar and complexes of ecologically contrasting taxa frequently occur (Carletto *et al*., 2009; Peccoud *et al*., 2009; Pifarretti *et al*., 2013). Morphological identification is also hampered by the high level of intraspecific variations. Indeed, the range of continuous morphological variation is probably wider in aphids than in any other insect group (Blackman & Eastop, 2000).

The presence of different morphs of aphids and their natural enemies further complicates species identification because they were morphologically similar (Foottit *et al*., 2008). Therefore, identification based on morphological traits alone may not be reliable and may be time consuming (Blackman & Eastop, 2000). This is even more difficult for the genus Aphis, thus so far, no taxonomist has succeeded in writing a comprehensive dichotomous morphological key that effectively separates all the species of a local fauna (Pifarretti *et al*., 2013). Furthermore, correct identification requires the taxonomist to have expertise in both entomology and botany. In this genus, some species can be identified on the basis of one easily distinguishable morphological character, but many are grouped within morphological entities known as ‘‘species groups’’. These ‘‘species groups’’, which have no taxonomic validity, bring species together that are difficult to tell apart morphologically (Cocur d’acier *et al*., 2007). Using this approach, only specimens for which an accurate host-plant association is available can be correctly identified. The morphological identification of winged morphs is reliable for only a fraction of the specimens caught in traps (Piffaretti *et al*., 2013).

2.4.2 DNA barcoding

DNA barcoding is based on the use of a short-standardised sequence to characterise species (Hajibabaei *et al*., 2006). For the case of insects, the mitochondrial cytochrome oxidase subunit 1 (COI) gene markers are used for insect molecular identification. The gene fragments are used to infer phylogenies particularly at a selected region at the 650 base pair fragments of the 5'- end of mitochondrial cytochrome c oxidase subunit I (COI) (Hebert *et al*., 2003). Species identification is achieved by comparing the sequence of an unknown sample to a reference database (Altschul *et al*., 1990). DNA barcoding is a molecular approach to species identification and is also being applied in pest monitoring and quarantine enforcement (Foottit *et al*., 2010). It is also a useful method in the study of insect epidemiology (Jalalizand *et al*., 2012). Sequencing of genome conservative regions like the mitochondrial COI gene and ribosomal DNA can be used for identifying insect populations with high polymorphism (Foottit *et al*., 2008; Hebert *et al*., 2003). DNA barcoding aims to identify species, as accurately, and faster than a taxonomist. It requires the use of an appropriate DNA marker with an adequate rate of evolution, and the availability of a reference dataset representative of the taxonomic diversity of the group studied. Even in a group for which extensive taxonomic studies have been carried out, such as aphids (Miller & Foottit, 2009), there are probably unidentified species and DNA barcoding, allowing the rapid detection of deep intraspecific barcode divergences, and this may facilitate the choice of interesting species for future taxonomic works (Hajibabaei *et al*., 2007).

DNA barcoding with the 5'-terminal fragment of the mitochondrial cytochrome C oxidase subunit 1 gene (COI) has proved to be an effective standardised approach for the characterization of diverse organisms (Hajibabaei *et al*., 2007; Hebert *et al*., 2003), including insects (Virgillo *et al*., 2010). Most DNA barcoding-based studies in insects have involved comparisons of small numbers of economically important species (Foottit *et al*., 2009; Kim *et al*., 2010; Shufran & Puterka, 2011; Wang & Qiao, 2009). Only a few recent studies have included relatively large numbers of species. Wang *et al*. (2011) focused on subtribe Aphidina, a difficult group, and two studies demonstrated the utility of DNA barcoding for the identification of specimens from the large regional insect fauna of North America and Korea (Foottit *et al*, 2008, 2009; Lee *et al*., 2011). In this study, the major focus will be to correctly identify aphids in dolichos and their associated parasitoids of agricultural importance in Njoro and Rongai. DNA barcoding of the parasitoids will, therefore, generate a reliable database on specimens earlier identified by a taxonomist.

CHAPTER THREE

EFFECT OF FIELD MARGIN VEGETATION DIVERSITY AND CROPPING SYSTEMS ON BEAN APHIDS AND THEIR NATURAL ENEMIES IN DOLICHOS (*Lablab purpureus* L.)

Abstract

Bean aphids are major insect pests accounting for 40% to 90% yield loss in most legumes. Management of these insect pests relies on synthetic insecticides which are expensive to farmers, causing environmental pollution and insect resistance. A study was conducted to determine the effect of the cropping system and diverse field margin vegetation in supporting the abundance and diversity of invertebrate taxa and the management of bean aphid infestation in dolichos beans. Field studies were conducted on farmers' fields in Njoro and Rongai sub-counties of Nakuru County, during the 2019 and 2020 long rains cropping seasons. The treatments consisted of dolichos monocrop and maize-dolichos intercrop planted in plots with field margin vegetation on either side or along one side. The treatments were arranged in randomised complete block design with eight replications (farms) in each sub-county. Data was collected on invertebrate taxa, bean aphid natural enemies' groups, bean aphid infestation and grain yield. Results showed that there was a total of eighteen (18) perennial species (8 in Njoro and 10 in Rongai). There were more weed species in Rongai (17%) compared to Njoro (14%) and more perennial weeds (21%) compared to annual weeds (13%), respectively. During the 2019 cropping season, the identified parasitoids from the barcodes were all primary parasitoids from *Aphidius* spp with *Aphidius colemani* being the only identified to species level. The invertebrate taxa family groups that were collected belonged to the Hymenopteran, Diptera, Hemiptera, Coleopterans and Orthopterans families. During the 2020 cropping season the barcoded primary parasitoids identified included the *Aphidius* sp., *Aphidius colemani*, *Lysiphlebus fabarum* and *Lysiphlebus* sp. Across the two cropping seasons. Results also showed that monocropped dolichos were more severely damaged by bean aphids compared to intercropped dolichos. Rongai had a higher land equivalent ratio 1.29 ,1.24 for 2019 and 2020, respectively compared to Njoro 1.24, 0.80 for 2019 and 2020, respectively. These findings indicate that inclusion of field margins around smallholder farms enhances the conservation of invertebrate taxa diversity and abundance which is an important functional mechanism of integrated pest management.

3.1 Introduction

Intensification of agriculture has resulted in increased use of input such as fertilisers, pesticides, irrigation and heavy machinery (Hazell & Wood 2008; Tscharntke *et al*., 2005). Agricultural expansion and intensification have largely contributed to loss of biodiversity and climate change (Foley *et al*., 2011; Rockstrom *et al*., 2009; Schneider *et al*., 2015). To sustain high crop yields in agricultural production more natural habitats are destroyed yearly to create space for agricultural landscapes (Tscharntke *et al*., 2005). Research findings have shown that agricultural intensification reduces yields because it disrupts natural pest regulation and crop pollination (Pimentel & Peshin, 2014; Potts *et al*., 2016). Invertebrates are very important service providers in an ecosystem as natural enemies and pollinators but are negatively affected by agricultural intensification because they need undisturbed habitats to persist in agro ecosystems for their continual existence (Holland *et al*., 2016; Potts *et al*., 2016; Rusch *et al*., 2016).

Landscape composition and habitat type are directly proportional to invertebrate taxa population since they regulate the invertebrate taxa communities and stabilise sustainable pest management by natural enemies in agro ecosystems. Simplified landscapes with monoculture farming systems have been found to reduce farmland biodiversity and biological control agents such as parasitoids and predators (Sorribas *et al*., 2016). Invertebrate taxa increase in diversity and numbers is achieved by habitats providing prey, food, refuge and hibernation. In return, invertebrates provide additional services like increasing pollination of the main crop and the efficacy of biocontrol (Meehan *et al*., 2012). High hedgerow cover in the surrounding area has been found to increase aphid parasitism (from 12 to 18%) and potential pollination (visitation rate and seed set increased by up to 70%) irrespective of local margin quality (Matteo *et al*., 2016). In farmlands in Kenya, field margin weed strips and fence hedgerows which provide shelter and alternate food sources for the natural enemies and pollinators are fast diminishing because of agricultural intensification through use of heavy machinery, frequent tillage, use of broad-spectrum pesticides and herbicides (FAOSTAT, 2017). Such operations normally disrupt invertebrate taxa communities, because they are carried out in the main crop fields without consideration of leaving refuge areas and floral resources for invertebrate taxa communities, mostly the natural enemies which require shelter during such agricultural operations (Heimpel *et al*., 2005). In farms where there is an arrangement of weed species strips and hedgerows with floral resources the rate of parasitism by natural enemies on pests and crop pollination is normally higher. Likewise, addition of non-crops has no negative impact on the main crops as they grow on unused areas. These hedgerows and weed margin strips act as banker plants which release the natural enemy when pest populations increase in crop fields and conserve them after harvest (Woltz *et al*., 2012).

Bean aphids are considered as a limiting factor in dolichos production in Kenya (Maluki & Akuja, 2019). It is considered as a major economic pest attacking dolichos causing yield loss of 20-40%. However, dolichos is commonly grown for its edible beans and leaves and is considered as an underutilised crop with immense potential. It is an important source of cheap dietary proteins compared to other animal proteins for the low-income communities in Kenya (Semba *et al*., 2021). Dolichos farming is popular particularly in the western, central and eastern regions of Kenya. The mean yield of dolichos bean in Kenya is 720 kg ha^{-1} with a potential of 2500-3000 kg ha^{-1} when biotic and abiotic stresses are addressed (Romeo *et al*., 2016). Bean aphids cause direct damage to dolichos leaves by sucking its sap (Yankova *et al.*, 2021). The phloem sap of dolichos contains high carbohydrates, proteins and amino acids content which are the main target for bean aphids (Ribeiro *et al*., 2018). Bean aphids secrete saliva that potentially interacts with defense mechanisms located in the sieve tube. This leads to loss of nutrients, disturbance of the translocation process and infection by viruses (Will *et al*., 2013). An infested plant shows symptoms such as leaf curling and distortion, especially young leaves at the centre of the crown, leaf yellowing and wilting, initially along the edges. The honey dew produced develops into black sooty mould. The pest indirectly causes damage through transmission of bean yellow mosaic virus (Al-Ani & Adhab, 2013).

Several methods have been deployed in the management of bean aphids which involves integrating chemical, and cultural control. Chemical control is however the most practised method by farmers (Laizer *et al*., 2019) in controlling bean aphids because it offers a quick response by keeping the pest population below damage thresholds. It is aimed at minimising initial colonisation and establishment and slowing the rate of increase once established. However, it has many limitations such that the molecules on the chemical are nonspecific and cause direct sub-lethal effects on natural enemies hence favouring pest outbreaks. There is a need to conserve natural enemies for natural pest regulation through the use of field margin vegetation and diverse cropping systems in the management of bean aphids.

Natural enemies: consisting of predators, parasitoids, pathogens, and weed feeders are invertebrate taxa that are key elements for biological control. These agents need to be conserved as biological control agents by means of habitat management in a smart way to achieve a sustainable pest management system, hence achieving the highest agricultural productivity. This ecological based approach provides favourable conditions for natural enemies for their fecundity, longevity, and access to pollen, nectar or sugar (Jacob & Evans, 2000). If an agroecosystem is favourable for natural enemies, they increase their population which makes them more successful in pest control. Generally, plant resources such as flowers are limited in agricultural farmlands in Kenya. Therefore, there is a need to manipulate the cropping system to allow for growing hedgerows and weed margin strips around crop fields. The technology will add more flowering plants which provides increased pollen and nectar for the natural enemy survival, subsequently leading to a large number of natural enemies conserved and available for natural pest regulation. Studies have shown evidence to suggest that semi-natural habitats in agricultural landscapes, can enhance pest control although they may also favour the colonisation of fields by pests (Bartual *et al*., 2019). Field margin vegetation offers food in the form of nectar and pollen and shelter for most adult predators and parasitoids (Holland *et al*., 2016).

Plant species diversity influences the natural enemies' abundance in an agro ecosystem. The type of field margin hedgerow around the farmland directly influences the diversity and abundance of insects that they attract including pests that may be of concern in adjacent crops (Pease & Zalom, 2009). The most important plant characteristics that greatly influences the abundance of natural enemies are the; period of blooming, floral area, maximum flower height (Jonhson *et al.*, 2010), hue, chroma, corolla size (Begum *et al*., 2004) and number of open flowers. Access to nectar and sugar largely depends on plant characteristics. When natural enemies such as parasitoids have more access to nectar and sugar, they have a higher percentage of fecundity and longevity of adult parasitoids (Khan *et al*., 2008). Predators mainly rely on prey resources for their growth and development. However, it is important to include phloem fluid and pollen in their non-prey diet to increase their fecundity and longevity (Hoki *et al*., 2014). The adult stage of many insects with predaceous larvae, like, green lacewings and syrphid flies and many adult parasites feed only on pollen and nectar.

On the other hand, many cropping systems practised by most farmers have an effect on pest population dynamics. Dolichos monocropping is practised by many farmers mainly because of compatibility with modern agricultural technologies (Altieri *et al*., 2012). Beans aphids are considered as a multivoltine pest that can cause a devastating effect on dolichos crop (Smith *et al*., 2021). Intercrop has been reported to reduce the population of herbivore species under a wide range of conditions. Intercropping interferes with the searching ability of a given pest for its host crop. A diverse cropping system provides shelter and alternative food sources for insect predators and parasitoids (Haber *et al*., 2021). The loss of these habitats leads to low-diversity, simplified landscapes that change the invertebrate community in ecosystems, which in turn affects the manner in which natural pest control works (Bianchi *et al*., 2006). Effective interventions are urgently needed to boost food production in agricultural environments in a sustainable manner, preserve biodiversity, and boost the number of natural enemies (Amoabeng *et al*., 2020; Ratnadass *et al*., 2012). Destruction of natural crop habitat and continuous monocropping has resulted to simplified landscapes with very little plant biodiversity which has led to interference of invertebrate composition in ecosystems, hence interfering with the natural functioning of natural pest management (Balmer *et al*., 2013; Morandin *et al*., 2014; Tscharntke *et al*., 2005). Hence there is an urgent need to manage habitats in an efficient and sustainable manner that increases and maintains a given invertebrate species throughout the year in a particular ecosystem.

Presently there are a limited but growing number of studies that focus on how landscapes in farmlands affect interactions between natural enemies, herbivores and agricultural primary production whereby farming systems concepts are incorporated in biological control agents' conservation (Matteo *et al*., 2016). This study determined the influence of field margin vegetation in conservation of invertebrate taxa in dolichos monocrop and maize-dolichos intercrop in high and low plant diversity areas across farmlands of Nakuru County in Kenya. Additionally, the study was designed to identify the most diverse and abundant aphid natural enemy community composition, responsible for bean aphid management through morphological and DNA barcoding.

3.2 Materials and methods

3.2.1 Experimental sites

Field studies were carried out at two locations: Njoro and Rongai Sub-Counties Nakuru County (Figure 3.1). In Njoro the farms were located at 0^{o}42' S, 36^{o} 10' E at an altitude range of 2000 to 2500 metres (m) above sea level (m.a.s.l). The annual rainfall range is 1000 to 1250 millimetres (mm) and temperature range is 17° C to 30° C. In Rongai the farms were located at 0^{o}10' S, 35^{o} 51' E at an altitude range of 1480 to 1550 m.a.s.l. The annual rainfall ranges between 750 to 1000 mm. and temperature 19° C to 32° C. Soils in Njoro are well drained dark reddish clays, classified as Mollic Andosols and Rongai soils are well drained sandy clay loams, classified as Vitric Andosols (Jaetzold *et al*., 2012).

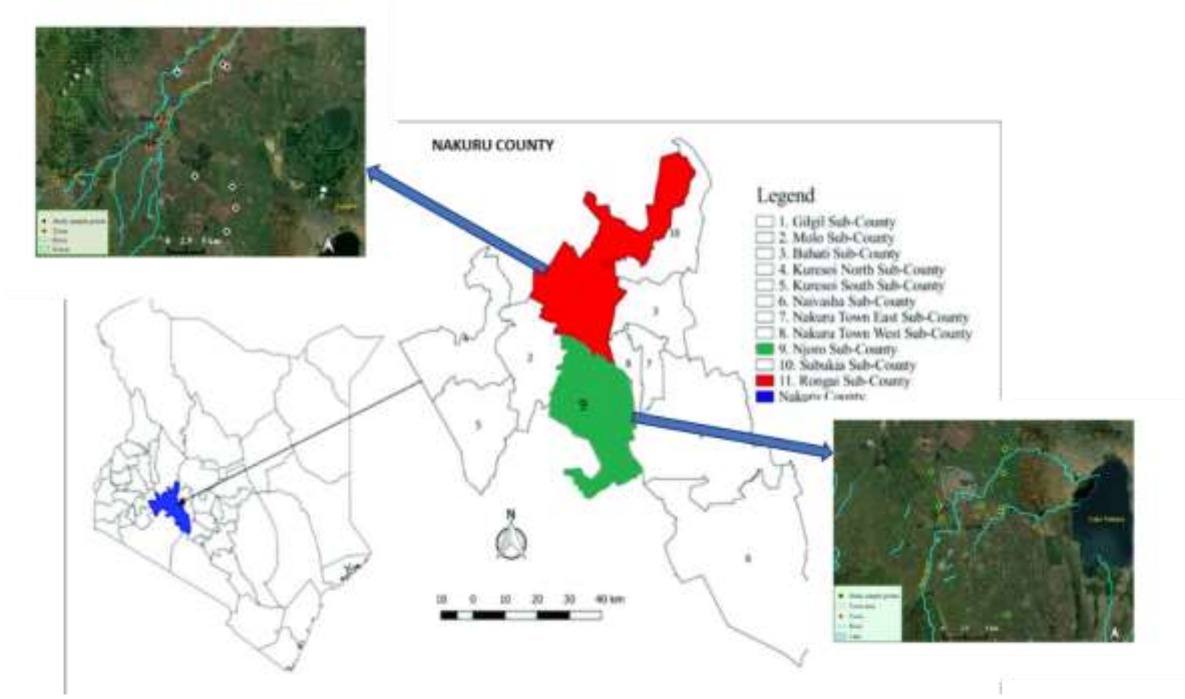

Figure 3. 1: Map displaying the study sites of Njoro and Rongai sub-counties.

3.2.2 Experimental procedure and design

The field experiment was conducted during May-December 2019 and March -November 2020 crop growing seasons. A total of sixteen farms; eight farms in Njoro and eight farms in Rongai were selected to represent different natural plant species diversity and abundance. The fields with more than ten (10) plant species and having at least 50% vegetation cover in the field margins were classified as high plant diversity (HPD) margins whereas fields with less than 10 plant species termed to be low plant diversity (LPD) margins.

The experiment was laid out in a randomised complete block design (RCBD) with each of the eight farms in a location being considered as a replicate. Dolichos monocrop and maize-dolichos intercrop were planted on plots measuring 10 m x 10 m with a natural field margins vegetation along at least two sides of the plot (Figure 3.2). Field margins in the study are defined as "whole of the crop edge, any vegetation strip present and the semi-natural habitat associated with the boundary". A compound fertilizer grade 23-23-0 (N.P.K) was used during planting which supplied 13.8 kg N/ha and 13.8 kg P_2O_5/ha as basal application. Planted crops were maize variety H516 obtained from Kenya Seed Company and dolichos variety DL1002 from KALRO Katumani Seed Unit. Dolichos monocrop was planted at a spacing of 60 x 30 cm, 2 seeds per hill, giving a plant population of 111,111 plants/ha. In the intercrop, maize was planted at a spacing of 75 x 30 cm, 1 seed per hill, giving a plant population of 44,444 plants/ha whereas dolichos was planted between maize rows at an intra-row spacing of 30 cm, 2 seeds per hill, giving a bean plant population of 88,888 plants. Top-dressing of maize was done at the seventh leaf stage using calcium ammonium nitrate (CAN) fertilizer at the rate of 46.2 kg N/ha. The fields were not sprayed with any pesticides during the whole experimental period.

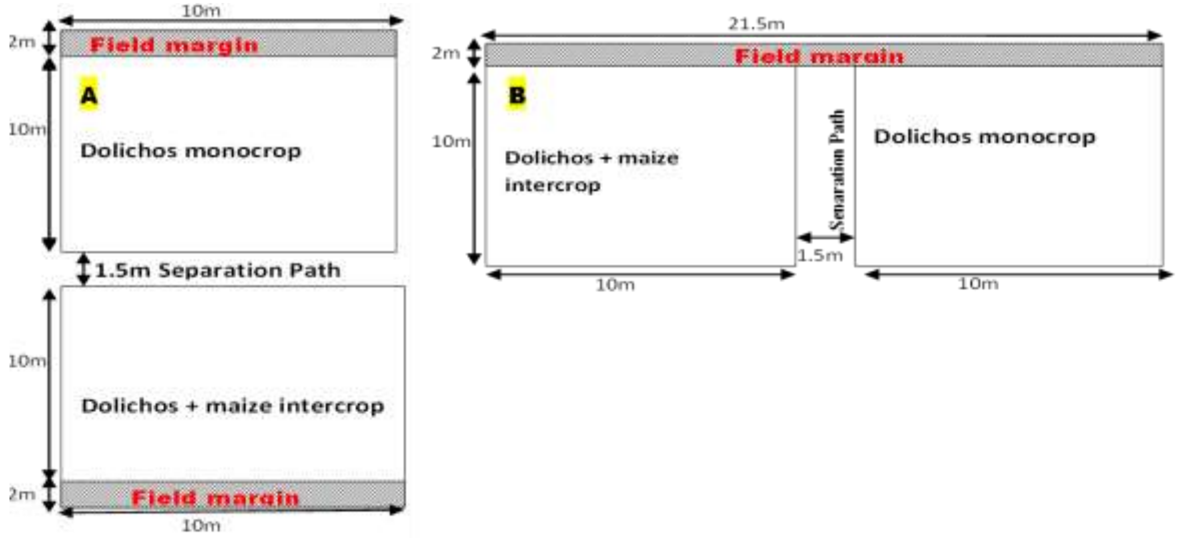

Figure 3. 2: Field experimental layout with either field margin vegetation on each side of the plots (A) or on one side of the plots (B) for on-farm trial.

3.3 Data collection

3.3.1 Field margin vegetation diversity and abundance

This was done by randomly throwing a 1 m^2 quadrat twice along the field margin vegetation when most of the field margin plant species were at flowering stage for ease of identification. All the individual field margin plants within the quadrat, were counted and separated according to species. The field margin plants were identified to species level using pictorial aids (e-library) and authentic identification was done by a plant taxonomist at the Department of Biological Science, Egerton University. The field margin vegetation and number of plants enclosed identified per species using plant taxonomic books were counted and recorded. Same plant species numbers from each quadrat were added and converted to percent cover per margin area. The plants were further classified as annual and perennial to assist in determining whether that affected the invertebrate taxa abundance trends, interaction and herbivore. The relative abundance of species of the field margin plants was calculated according to (Equation 3.2) and number of species in the community calculated as (Equation 3.3) (Mahajan & Fatima, 2017).

$$RAS\ (\%) = \frac{Number\ of\ specific\ species\ \ in\ the\ quadrat}{Total\ number\ of\ all\ species\ in\ the\ quadrat} \times\ 100 \qquad \text{Equation 3.1}$$

Where RAS = relative abundance of species

$$D = 1 - \frac{\sum n(n-1)}{N(N-1)} \qquad \text{Equation 3.2}$$

where: *D* = is the number of species in a community, n = the number of individuals displaying one species and N = the total number of all individual

3.3.2 Diversity and abundance of parasitoids

Sampling to determine the effect of diverse field margin vegetation on the diversity, abundance and temporal distribution of invertebrate taxa, was done using yellow pan trap, yellow sticky card and sweep netting simultaneously at three dolichos growth stages; vegetative, flowering and podding stage. Yellow traps were chosen to influence insect attraction through colour spectra.

a) Pan trapping

This was done according to Saunders and Luck (2013) whereby a yellow plastic pan measuring 20 cm diameter and 5 cm high was used to capture the ground dwelling natural enemies (Plate 3.1). The pans were filled with a premixed liquid solution containing 250 ml of water, 5 g of salt (sodium chloride) to preserve the natural enemies and 5 ml of odourless liquid detergent to break the surface tension of the water. Two pan traps were placed at the ground level, one at the centre of the experimental plot and another along the field margin vegetation. The traps were left in the field for 48 hours after which, the trapped insects were retrieved by sieving out the premix and washing them with clean water using a wash bottle. The insects were picked from the sieve using a camel hairbrush size 00 and placed in 50 ml plastic falcon tubes filled with 25 ml of 70% ethanol for preservation before being taken to the laboratory. The insects were placed under a dissecting microscope (Leica ZOOM 2000 Inc. Buffalo. NY U.S.A 14240-0123) at 200× magnification for counting and identification up to the family level using Simon and Schuster's identification keys (Arnett & Jacques 1981). This data collection procedure was repeated, early vegetative, late vegetative, and reproductive growth stages of dolichos.

Plate 3. 1: Yellow Pan trap at dolichos crop centre (A) and field margin vegetation (B).

b) Sticky trapping

Two yellow sticky cards measuring 8 cm width x 24 cm length were suspended on sticks 30 cm above the dolichos crop and field margin vegetation canopy to capture flying invertebrates (Melanie *et al*., 2015). One card was tied on a 30 cm stick and the stick placed at the centre of the crop plot and the other at the centre of the field margin vegetation. This was done at the same period as that of the pan traps during the cropping season. The sticky traps were retrieved 48 hours later from the fields, placed into non-sticky laminating pouches of 25 cm x 10 cm and taken to the laboratory for identification. The insects were removed from the sticky traps using hexane (85%) AR according to the method of Walsh *et al.* (2005). The sticky card was placed inside the 1 litre jars and thereafter 10 ml of hexane was poured, the jars locked for 1 minute to soak and dislodge the insects. The hexane solution was filtered out and subsequently, the specimens were conserved in 70% ethanol for identification up to family level.

Plate 3. 2: Yellow sticky trap placed at crop centre (A) and field margin vegetation (B).

c) Sweep netting

This was done above the field margin vegetation using a 40 cm diameter sweep net, according to Spafford and Lortie (2013). Ten sweepings were taken at an angle of 180° while walking for 5 m on a straight line, starting from the edge of the field margin to the centre (Plate 3.3). The 10 sweeps were considered as one complete sweep, which was referred to as one replicate sweep. This was repeated three times to achieve three replicates per field margin plot. Replicate lines were carried out 2 metres parallel from the previous sweep-line. The sweep net bag was closed immediately after sweeping. After a short while, the sweep net was carefully opened and insects manually sucked out using an aspirator. Then transferred to a jar containing 2 ml of formalin and left for 2 hours to die. Once inactive, they were transferred into 25 ml 70% ethanol in 50ml falcon tubes and taken to the laboratory for identification. All the collected specimens were identified to the family level using the taxonomic keys according to Simon and Schuster's identification keys (Arnett & Jacques, 1981).

Plate 3. 3: Sweep netting along field margin vegetation.

3.3.3 Abundance and diversity of bean aphid parasitoids

Sentinel plants were used for assessing aphid parasitization. These plants were potted dolichos seedlings that had 60 bean aphids (*Aphis fabae*) per plant. Sentinel plants had been placed into the experimental plots the same day that transparent sticky traps and pan traps were removed. Two sentinel plants were planted in each plot (dolichos monocrop or maize-dolichos intercrop); one was positioned in the middle of the crop (5 m from the outermost row), and the other was positioned in the margin vegetation (0.5 m from the outermost row). Aphid parasitoids laid their eggs on the plants by leaving them in the field plots for a period of seven days. The sentinel plants were placed in mesh cages after being recovered and covered with a fine net mesh throughout transportation. The number of live parasitoids that emerged, dead parasitoids, and aphid mummies was counted and recorded every three days for thirty days. Aphids reproduce quickly and can be difficult to find in big cages, so it was not practical to count the non-parasitized aphids. Instead, we concentrated on standardising the initial aphid population.

3.3.4 Molecular characterisation of bean aphid parasitoids

All the parasitoids collected through the aid of sentinel plants as in section 3.3.3 during the two seasons in Njoro and Rongai environments were further identified by mitochondrial cytochrome oxidase I (COI) barcoding and confirmed by sequencing ; (Plate 3.4). DNA extraction and sequencing. DNA was extracted from parasitoids using the Chelex method. Whole insects were ground in 25 µl of Chelex buffer [10% w/v Chelex resine (Bio-Rad, Hercules, USA) in TE solution] using a micropestle in a 1.5 ml Eppendorf tube. The micropestle was rinsed with a further 25 µl of Chelex buffer. A PCR was performed using the primers HCO2198 and LCO1490 (Folmer *et al.*, 1994) and MyTaq DNA polymerase (Meridian Bioscience, Cincinnati, USA) following manufacturer's instructions. The PCR conditions used were: Initial denaturation of 94 °C for 3 min, 37 cycles of 94 °C for 30 sec, 52 °C for 1 min, 72 °C for 1 min 30 sec and a final extension step of 72 °C for 10 min. Where this initial PCR was unsuccessful, LepF1/C_ANTMRID (Hebert *et al.,* 2004; Smith *et al.*, 2008) and MLepF1/LepR1 (Hajibabaei *et al.,* 2007; Hebert *et al.,* 2004) primers were used to amplify shorter sections of DNA (Smith *et al.*, 2008). PCR products were visualised on a 1.2% agarose gel in 0.5 x TBE stained with GelRed (Biotium, Fremont, USA) using gel electrophoresis. When amplification by PCR was successful, PCR products were purified using a GeneJET PCR purification kit (ThermoFisher Scientific, Waltham, USA) following manufacturer's instructions. The PCR product was sequenced by Eurofins Genomics (Eurofins, Luxembourg) using the forward primer.

Plate 3. 4: Parasitoids identification (A), isolation (B) and preparation (C) for DNA extraction.

3.3.5 Parasitoid DNA Barcoding

All DNA sequences were trimmed and quality checked using Geneious Prime 2020.0.5 (https://geneious.com). Both LepF1/C_ANTMRID and MLepF1/LepR1 primer pairs produced high quality sequences; a consensus sequence (full length COI barcode) was produced from these shorter sequences in Geneious Prime. Amplification with only one of LepF1/C_ANTMRID and MLepF1/LepR1 primer pairs was successful, this shorter sequence or ‘mini-barcode’ (Mkenda *et al*., 2019) was to identify the specimen, but not for further analysis. Sequences were compared to published sequences in the Barcode of Life Database (BOLD; http://www.boldsystems.org/) for identification. If the specimen sequence gave a close match (greater than 99% for species, greater than 95% for genus and greater than 90% for family) and the sequence clustered with a monophyletic group of this species/genus/family, then the unknown sequence was assigned to this taxon (Hebert *et al*., 2016).

3.3.6 Aphid abundance

Aphid abundance was determined by visually observing and scoring the level of infestation. A total of ten plants were randomly selected from the inner five rows in each treatment at seedling, early vegetative, late vegetative and flowering dolichos growth stages. The aphid abundance score was done using a categorical scale, whereby: 1 = no aphid infestation, 2 = a few scattered aphids (1-100), 3 = a few isolated colonies (101-300), 4 = several isolated colonies (301-600), 5 = large isolated colonies (601-1000) and 6 = large continuous colonies (>1001). (Aken *et al.,* 2013) (Table 3.1).

3.3.7 Aphid damage severity

This was determined by visually observing and scoring the level of aphid damage on ten randomly selected plants from the inner five rows in each experimental unit. The damage severity was done using a 1 to 5 scale, where; 1= no infestation or damage, 2=light damage and infestation < 25 % plant parts damaged or infested, 3=average damage and infestation 26 % - 50 % plant parts damaged, 4=high infestation and damage 51 % - 75 % plants parts damaged showing yellowing of lower leaves and 5= severe infestation >75 % damage resulting to plants with high infestation levels with a yellow and severely curled or dead plant (Mkenda *et al.*, 2015) (Table 3.2).

3.3.8 Percent aphid incidence

Percentage incidence score evaluation was assessed by walking in diagonal or a zigzag manner across the field and scoring 30 random plants for the presence or absence of bean aphids on dolichos bean. The number of plants infested by aphids were divided by the total of plants assessed (30 plants) and multiplied by one hundred to get the percentage incidence.

$$Percent\ incidence\ (\%) = \frac{N}{T} \times 100 \qquad \text{Equation 3.3}$$

where N = Number of infested plants, T=Total number of plants assessed

3.3.9 Dolichos bean grain yield and productivity of intercropping

Dolichos yield was determined in the whole plot excluding the two rows from the margin. The total yield from each plot was harvested and sun dried to a moisture content of 12% then weighed and converted into kilograms per hectare using equation 3.3.

$$\text{GY} = \frac{GWP \times 10}{HA\ (m^2)} \qquad \text{Equation 3.4}$$

where GY=Grain yield in tons/ha, GWP= Grain weight per plot and HA= Harvest area in m2 Intercropping productivity was assessed using land equivalent ratio (LER) equation below as described by Malèzieux *et al*. (2009) equation 3.6.

$$\text{LER} = \frac{Yia}{Ysa} + \frac{Yib}{Ysb} \qquad \text{Equation 3.5}$$

where, LER= land equivalent ratio; Yia= intercrop yield of maize; Ysa = yield of sole maize; Yib= yield of intercrop dolichos and Ysb = yield of sole dolichos.

Table 3. 1: Aphid abundance assessment scale.

Scale	Aphid abundance	Visual expression
1	None	
2	A few scattered aphids	
3	A few small isolated colonies	
4	Several small isolated colonies	
5	Large isolated colonies	
6	Large continuous colonies	

Source: Aken *et al*. (2013)

Table 3. 2: Damage severity scoring scale.

Scale	Description of damage	Visual expression
1	No symptoms of an attack	
2	Showing damage symptoms up to 25%	
3	Showing damage symptoms from 26-50%	
4	Plant showing yellowing of older leaves and curling of young leaves 51-75%	
5	Severely stunted plants with severely curled and yellow leaves and leaves covered with sooty mould or dead plant >75%	

Source: Mkenda *et al*. (2015)

3.4 Data analyses

Aphid severity, incidence and abundance were subjected to repeated measure analysis of variance using a general linear mixed model. (SAS, Institute, Cary NC, 2011). Prior to running ANOVA to carter for the zeros on plants that were found with no aphids. The data on the number of natural enemies, aphid population, aphid mummies, severity of damage and incidence were correlated after every sampling using R statistics. The results were used to define the most effective natural enemy in controlling bean aphid on dolichos under low and high plant diversity environments. Statistical model adopted for analysis described in equation 3.9.

The data on grain yield and the population dynamics of aphids were later subjected to Analysis of Variance (ANOVA) and means separated using Tukey's HSD test (Tukey, 1949). The number of parasitoids (including those for which barcodes were not obtained) emerged from sentinel plants was analysed using a GLM in R-Studio. The GLM had a poisson distribution with region and cropping type (intercrop or monocrop) included as covariates without interactions. Parasitoid specimen sequences and published reference sequences from BOLD were used to construct phylogenetic trees. Sequences were aligned in MEGAX (Kumar *et al*., 2018) using ClustalW, then any sequences that contained missing or ambiguous bases were removed.

The diversity of the natural enemies was determined using Shannon-Weaver index of diversity (H) and Pielou evenness index (E).

$$H = -\Sigma p_i ln p_i \qquad \text{Equation 3.6}$$

where H is the Shannon's diversity index and pi is the proportion of individuals found in the ith species ln is the natural log of individuals found in the ith species

$$E = H/ln\, ln\, S \qquad \text{Equation 3.7}$$

where E is the Pielou's Index of Evenness, H is the calculated Shannon Index ln (S) is the species diversity under maximum under equitability condition and S is the number of species in the community.

Field margin plant species diversity was determined using the Simpsons' species diversity index.

$$D = 1 - \frac{\Sigma n(n-1)}{N(N-1)} \qquad \text{Equation 3.8}$$

where n is the number of individuals displaying one species and N is the total number of all individuals.

$$Y_{ijklm} = \mu + L_i + F_j + LF_{(j)i} + T_k + FT_{ik} + LT_{ik} + LFT_{i(j)k} + S_l + LS_{il} + TS_{kl} + LTS_{ikl} + LFTS_{i(i)kl} + \varepsilon_{ijklm}$$

Equation 3.9

where;

Y_{ijklm} = Response variable; μ = Overall mean; L_i= Effect due to ith location; F_j = Effect due to jth farmer (replicate); $F_{(i)j}$ = Interactive effect of the jth farmer (replicate) within the ith location; $T_{(j)k}$ = Effect due to kth treatment; $FT_{(i)k}$ = Interactive effect of the jth replicate in the kth treatment; LT_{ik} = Interactive effect of the kth treatment in the jth location; $LFT_{(i)jk}$ = Interactive effect of the jth treatment in the kth replicate within the jth location; S_l = Effect due to lth season; LS_{jl} = Interactive effect of the lth season in the ith location; TS_{kl} = Interactive effect of the lth season in the kth treatment; $LTS_{(i)jl}$ = Interactive effect of the lth season on the kth treatment within the ith location; $LFTS_{i(i)kl}$ = Interactive effect of the lth season on the kth treatment in the jth replicate within the ith location location; ε_{ijklm}= Random error term to test the model.

3.5 Results

3.5.1 Field margin type and abundance in Njoro and Rongai farmers field during 2019 and 2020 cropping season

The ANOVA results revealed that the location had a significant effect on species richness, total abundance and Simpson diversity index. The interaction between growth habitat and location had a significant effect on species richness and total abundance while the interaction between season and location had a significant effect on total abundance (Table 3.3).

Table 3. 3: Analysis of variance for species richness, total abundance and Simpson diversity index (SDI).

Source of variation	**Species Richness**	**Total Abundance**	**Simpson diversity index (SDI)**
R^2	0.618	0.387	0.560
F	2.845	1.113	2.245
Pr > F	<0.0001	0.333	0.001
Location	0.005	0.113	1.106
	0.945	0.737	0.296
Season	0.798	0.034	2.426
	0.374	0.854	0.123
Farmer (REP)	1.139	0.107	1.240
	0.348	0.998	0.291
Treatment	0.383	0.168	0.028
	0.538	0.683	0.867
Growth habitat	88.678	21.116	54.429
	<0.0001	<0.0001	<0.0001
Location×Season	1.063	0.066	0.632
	0.306	0.797	0.429
Location×Farmer	0.933	0.121	1.583
	0.486	0.997	0.152
Location×Treatment	0.005	0.168	0.114
	0.945	0.683	0.737
Location×Growth habitat	5.788	19.907	1.450
	0.018	<0.0001	0.232
Season×Farmer	0.151	0.133	0.286
	0.993	0.996	0.958
Season×Treatment	0.005	0.030	0.142
	0.945	0.863	0.707
Season×Growth habitat	0.005	4.889	3.321
	0.945	0.030	0.072

Table 3.3: Continued…

Source of variation	**Species Richness**	**Total Abundance**	**Simpson diversity index (SDI)**
Location×Season×Farmer	1.797	0.125	2.023
	0.099	0.996	0.062
Location×Season×Treatment	2.084	0.049	0.074
	0.153	0.825	0.786
Location×Season×Growth habitat	0.005	0.013	0.058
	0.945	0.911	0.810
Location×Farmer×Treatment	0.562	0.175	0.509
	0.785	0.990	0.825

F= f-value, Pr>F= P value

Results on the means for Species type, abundance and Simpson's diversity index of field margin vegetation on the two locations revealed a more abundant annual field vegetation species in Njoro (66.25) compared to Rongai (44.25) and a more abundant perennial field margin vegetation in Rongai (54.37) compared to Njoro (31.56) (Table 3.4). There was no significant difference on the species richness, abundance and field margin vegetation on both monocrop and intercrop during the two seasons (Table 3.5).

Table 3. 4: Species richness, abundance and Simpson's diversity index for annual and perennial plants in field margin vegetation in Njoro and Rongai during 2019 and 2020 cropping seasons.

Location	May-December 2019 cropping season					
	Annual plant species			**Perennial plant species**		
	Species richness	Species abundance	SDI	Species richness	Species abundance	SDI
Rongai	3.87±0.34^{a}	44.25±6.96^{b}	0.64±0.05^{a}	2.25±0.23^{a}	54.37±6.86^{a}	0.31±0.04^{a}
Njoro	4.62±0.37^{a}	66.25±5.17^{a}	0.66±0.02^{a}	1.93±0.23^{a}	31.56±4.52^{b}	0.27±0.06^{a}
MSD	1.05	13.40	0.13	0.57	13.10	0.16
	March-November 2020 cropping season					
Rongai	3.87±0.36^{a}	55.43±6.91^{a}	0.53±0.05^{a}	2.31±0.29^{a}	43.93±6.89^{a}	0.37±0.06^{a}
Njoro	4.18±0.37^{a}	75.93±6.91^{a}	0.51±0.05^{a}	1.50±0.25^{a}	17.31±4.54^{b}	0.24±0.06^{a}
MSD	1.20	20.67	0.17	0.84	16.36	0.20

Means in a column followed by the same letters are not significantly different using Tukey's HSD test at $P < 0.05$; SDI-Simpson's Diversity Index.

Table 3. 5: Species richness, abundance and Simpson's diversity index of field margin vegetation in dolichos monocrop and maize-dolichos intercrop for Rongai and Njoro during 2019 and 2020 cropping seasons.

Location	**May-December 2019 cropping season**					
	Dolichos monocrop			**Maize-Dolichos intercrop**		
	Species richness	Species abundance	SDI	Species richness	Species abundance	SDI
Rongai	3.31±0.37^{a}	49.43±6.95^{a}	0.48±0.06^{a}	2.81±0.33^{a}	49.18±7.11^{a}	0.47±0.06^{a}
Njoro	3.18±0.45^{a}	50.00±6.10^{a}	0.47±0.07^{a}	3.37±0.47^{a}	47.81±7.06^{a}	0.46±0.07^{a}
MSD	1.01	21.81	0.16	0.95	23.27	0.13
	March-November 2020 cropping season					
Rongai	3.00±0.34^{a}	41.56±7.34^{a}	0.42±0.05^{a}	3.18±0.43^{a}	49.81±6.77^{a}	0.47±0.06^{a}
Njoro	3.06±0.49^{a}	49.75±9.00^{a}	0.37±0.07^{a}	2.62±0.44^{a}	43.50±9.92^{a}	0.37±0.06^{a}
MSD	1.05	23.81	0.17	1.10	23.18	0.20

Means in a column followed by the same letters are not significantly different using Tukey's HSD test at $P < 0.05$; SDI-Simpson's Diversity Index

The annual field margin vegetation abundance was 100 for all the cropping seasons in Njoro and Rongai locations. Njoro recorded a species rich location (11) during the 2019 cropping season similarly in 2020 cropping season was higher (15) than Rongai (Table 3.6). *Galinsoga parviflora* (17.88%) and *Brassica napus* (49.13%) were the most abundant annual species in Njoro and Rongai locations, respectively during the 2019 cropping season (Table 3.6). Similarly, *Bidens pilosa* (21.3%) and *Eleusine indica* (18.94%) were the most abundant annual species in Njoro and Rongai locations, respectively during the 2020 cropping season (Table 3.6).

Table 3. 6: Relative abundance of annual field margin vegetation in Rongai and Njoro during 2019 and 2020 cropping seasons.

		2019		**2020**	
Common name	**Scientific name**	**Njoro**	**Rongai**	**Njoro**	**Rongai**
Amaranthus	*Amaranthus retroflexux*	6.66	.	4.36	3.69
Black jack	*Bidens Pilosa*	15.48	.	23.1	6.05
Black night shade	*Solanum nigrum*	.	3.18	.	.
Double thorn	*Oxygonum sinuatum*	.	.	1.89	4.72
Fat hen	*Chenopodim album*	.	.	1.63	.
Fleabane	*Conyza bonaviensis*	5.94	.	.	12.15
Gallant soldier	*Galinsoga parviflora*	17.88	.	15.36	11.45
Goat weed	*Ageratum conyzoides*	.	.	6.75	5.78
Lion's ear	*Leonotis nepetifolia*	.	.	7.54	2.46
Love grass	*Eragrotis curvula*		14.74	.	.
Mexican marigold	*Tagetes minuta*	12.44	.	6.03	7.21
Oxalis	*Oxalis latifolia*	5.67	13.3	4.34	.
Rattle pod	*Crotalaria lanceolata*	.	.	5.64	7.7
Thorn apple	*Datura stramonium*	6.89	19.64	5.29	2.71
Wandering jew	*Commelina bengalensis*	6.73	.	5.89	3.35
Wild finger millet	*Eleusine indica*	7.31	.	2.41	18.94
Wild rape seed	*Brassica napus*	1.22	49.13	1.3	13.8
Wild vetch	*Vicia sativa*	13.79	.	8.47	.
Plant Species Richness		11	5	15	13

The perennial field margin vegetation abundance was 100 for all the cropping seasons in Njoro and Rongai locations. Rongai recorded a species rich location (13) during the 2020 cropping season (Table 3.7). *Chloris gayana* was the most abundant perennial plant species in both Njoro (31.38%) and Rongai (31.31%) during the 2019 cropping season (Table 4.7). *Parthenium hysterophorus* (26.19%) and *Cynodon dactylon* (18.41%) were the most abundant perennial field margin species in Njoro and Rongai location, respectively during the 2020 cropping season (Table 3.7).

Table 3. 7: Species abundance (%) of perennial field margin vegetation in Rongai and Njoro during 2019 and 2020 cropping seasons.

		2019		2020	
Common name	**Scientific name**	**Njoro**	**Rongai**	**Njoro**	**Rongai**
African basil	*Ocimum gratissimum*	1.51	.	.	.
Bitter-broom	*Parthenium hysterophorus*	.	.	26.19	.
Chinese lantern	*Physalis angulata*	8.49	19.55	.	3.37
Couch grass	*Cynodon dactylon*	.	13.89	16.33	18.41
Dandelion	*Taraxacum officinale*		.	.	16.08
Devil's horse whip	*Achyranthes aspera*	14.12	2.88	24.49	1.05
Gooseberry	*Physalis peruviana*	.	.	.	2.64
Khaki weed	*Alternanthera punges*	.	.	.	2.95
Kikuyu grass	*Pennisetum clandestinum*	3.53	.	.	.
Lantana camara	*Lantana camara*	9.41	.	.	.
Love grass	*Eragrotis curvula*	9.61	7.61	16.33	4.49
Nut grass	*Cyperus rotundus*	9.41	3.78	.	.
Poverty grass	*Danthonia spicata*	.	.	16.67	11.07
Rhodes grass	*Chloris Gayana*	31.38	31.31	.	12.21
Sodom apple	*Solanum incanum*	.	.	.	1.11
Sow thistle	*Sonchus arvensis*	.	8.50	.	.
Star grass	*Cynodon plectostachyus*	.	.	.	5.48
Verbena	*Verbena bonaviensis*	.	1.98	.	
White eye	*Richardia brasiliensis*	.	.	.	17.32
Wire grass	*Eriachne obtusa*	12.54	10.49	.	
Total abundance		100	100	100	100
Species richness		9	9	5	13

3.5.2 Bean aphids natural enemies species richness, abundance, diversity and evenness as influenced by cropping season, location (environment), crop growth stage and cropping system.

The ANOVA results showed that cropping season had a significant (*P*<0.001) effect on species richness, abundance and index (Table 3.8). The effect of location was significant species richness, abundance, index and evenness. The crop growth stage and cropping season

(*P*<0.0001) affected the species abundance. The interaction between season and location was significant in the species richness, abundance and diversity. The interaction between season and cropping system was significant in the species abundance. The interaction between season, location, growth stage and cropping system was significant on species richness, abundance and diversity (Table 3.8).

Table 3. 8: ANOVA for species richness, abundance, index and evenness.

Source of variation	**df**	**Species Richness**	**Species abundance**	**Species diversity**	**Species evenness**
Season	1	15.02***	1.10***	203.01***	137.19***
Location	1	1.75*	0.05*	0.43**	2.26**
Season × Location	1	2.36**	0.01*	0.32**	1.83
Crop growth stage	3	0.16	0.06**	0.33*	0.06
Season × growth stage	2	0.07*	1.19**	1.20	0.06
Location × growth stage	3	0.34*	0.17	0.15	0.87
Cropping system	1	0.16	1.44	0.02**	0.13
Season × Cropping system	1	0.33	1.07**	0.74	2.08
Location × Cropping system	1	0.00	0.03*	0.00	0.28
Growth stage × Cropping system	3	0.20*	0.19	0.32	0.94
Season × Location × Growth stage × Cropping system	10	0.30**	0.32*	0.09*	1.13
R^2		**0.54**	**0.41**	**0.82**	**0.78**
CV		**19.7**	**21.71**	**20.27**	**18.51**

*, **, ***, significant at significant at (*P*≤0.05), (*P*≤0.01), (P≤0.001), respectively; CV=Coefficient of variation; d.f= degree of freedom.

During the 2019 cropping season, in Njoro, the highest species richness was observed at flowering stage (7.2) on dolichos monocrop. In Rongai, the highest species richness was observed on maize-dolichos intercrop (6.2) at vegetative stage. Species abundance was highest in maize-dolichos at flowering (17.7) and lowest in dolichos monocrop at vegetative growth stage (9.8) in Njoro. In Rongai the highest species abundance was observed in dolichos monocrop at flowering (30.9) and lowest in maize-dolichos intercrop at podding (12). Species diversity index in Njoro was highest at vegetative and flowering stages (0.9) on maize-dolichos intercrop and lowest in dolichos monocrop at pooding stage (0.4). In Rongai the dolichos monocrop at vegetative stage had the highest species diversity (0.9) and lowest at vegetative stage for dolichos monocrop (0.4) and maize-dolichos intercrop (0.5). Species evenness in

Njoro was highest in maize-dolichos intercrop at vegetative stage (0.5) and lowest in dolichos monocrop at podding (0.1). In Rongai the highest species evenness was observed in maize-dolichos intercrop at podding (0.5). In dolichos monocrop the lowest species evenness was observed at vegetative and budding stages (0.3) (Table 3.9).

During the 2020 cropping season, in Njoro, the highest species richness was observed on dolichos monocrop at flowering stage (4.2). In Rongai, the highest species richness was observed on dolichos monocrop at podding stage (5.6). In Njoro species abundance was highest in dolichos monocrop at flowering (18.1) and lowest in maize-dolichos intercrop at podding stage (14). In Rongai the highest species abundance was observed in dolichos monocrop at flowering (18) and lowest in maize-dolichos intercrop at flowering (13.7). Species diversity index in Njoro was highest at flowering stages for dolichos monocrop and maize-dolichos intercrop (o.8). The lowest Species diversity index was observed in maize-dolichos intercrop at vegetative stage (0.6). In Rongai the maize-dolichos intercrop and dolichos monocrop at flowering stages had the highest species diversity (0.7). The lowest Species diversity index was observed at vegetative and podding stages for maize-dolichos intercrop and at podding stage for dolichos monocrop (0.4). Species evenness in Njoro was highest on dolichos monocrop at vegetative stage (0.5) and lowest in maize-dolichos intercrop at podding (0.1). In Rongai the highest species evenness was observed in maize-dolichos intercrop at flowering (0.5). The lowest species evenness was observed in dolichos monocrop at flowering stage (0.2) (Table 3.9).

Table 3. 9: Mean species richness, abundance, diversity and evenness on species as influenced by cropping season, location, crop growth stage and cropping system.

	Species Richness					
	Monocrop			Intercrop		
2019 Season	**Vegetative**	**Flowering**	**Podding**	**Vegetative**	**Flowering**	**Podding**
Njoro	4.6^{b}	7.2^{a}	6.5a	5.9b	5.7b	5.6a
Rongai	6.0^{a}	6.2^{b}	4.6b	6.2a	6.1a	5.4a
Hsd$_{0.05}$	0.4	0.1	0.3	0.1	0.2	0.4
2020 Season						
Njoro	3.4^{b}	4.2^{b}	2.9^{b}	3.8^{b}	3.6^{b}	3.0^{b}
Rongai	4.5^{a}	5.1^{a}	5.6^{a}	4.4^{a}	4.4^{a}	5.0^{a}
Hsd$_{0.05}$	0.1	0.2	0.4	0.3	0.2	0.3

	Species Abundance					
	Monocrop			Intercrop		
2019 Season	**Vegetative**	**Flowering**	**Podding**	**Vegetative**	**Flowering**	**Podding**
Njoro	9.8^{b}	43.0^{a}	21.2^{a}	12.6^{b}	17.7^{b}	12.2^{a}
Rongai	26.4^{a}	30.9^{b}	14.9^{b}	16.0^{a}	24.0^{a}	12.0^{a}
Lsd$_{0.05}$	7.1	4.2	5.5	1.2	3.7	0.2
2020 Season						
Njoro	17.0^{a}	18.1^{a}	10.8^{b}	16.4^{a}	16.9^{a}	14.0^{b}
Rongai	15.5^{b}	18.0^{a}	17.0^{a}	15.8^{b}	13.7^{b}	17.7^{a}
Hsd$_{0.05}$	0.3	0.2	0.4	0.3	0.6	0.5

Means in a column followed by the same letters are not significantly different using Tukey's HSD test at $P < 0.05$.

Table 3.9: Continued…

	Species diversity					
	Monocrop			Intercrop		
2019 Season	**Vegetative**	**Flowering**	**Podding**	**Vegetative**	**Flowering**	**Podding**
Njoro	0.5a	0.6b	0.4b	0.9a	0.9a	0.7a
Rongai	0.4a	0.9a	0.8a	0.5b	0.8a	0.8a
Hsd$_{0.05}$	0.2	0.1	0.3	0.2	0.2	0.3
2020 Season						
Njoro	0.7a	0.8a	0.7a	0.6a	0.8a	0.7a
Rongai	0.5a	0.7a	0.4b	0.4b	0.7a	0.4b
Hsd$_{0.05}$	0.2	0.3	0.1	0.1	0.1	0.2
	Species evenness					
	Monocrop			Intercrop		
2019 Season	**Vegetative**	**Flowering**	**Podding**	**Vegetative**	**Flowering**	**Podding**
Njoro	0.4^{a}	0.2^{b}	0.1^{b}	0.5^{a}	0.2^{a}	0.4^{a}
Rongai	0.3^{a}	0.4^{a}	0.3^{a}	0.4^{a}	0.3^{a}	0.5^{a}
Hsd$_{0.05}$	0.1	0.0	0.1	0.1	0.1	0.1
2020 Season						
Njoro	0.5^{a}	0.3^{a}	0.2^{b}	0.2^{a}	0.4^{a}	0.1^{b}
Rongai	0.4^{a}	0.2^{a}	0.4^{a}	0.3^{a}	0.5^{a}	0.4^{a}
Hsd$_{0.05}$	0.1	0.1	0.0	0.1	0.1	0.2

Means in a column followed by the same letters are not significantly different using Tukey's HSD test at $P < 0.05$.

3.5.3 Bean aphid natural enemies' populations as influenced by cropping season, crop growth stage and cropping system in Njoro.

During the 2019 cropping season, a total of 2210 individual natural enemies were captured on farmer's fields from five orders and twelve families. The natural enemies are classified as either predators or parasitoids. Parasitoids consisted of the majority of natural enemies with 82.5% of the total collection during this period. The order Diptera had the highest relative abundance of 75% and lowest in Hemiptera at 1.3%. Among the families identified, the highest relative abundance was observed in Syriphidae (30.9%) and lowest in Geocoridae (0.5%) (Table 3.10). In relation to the cropping system and dolichos growth stage, Syriphidae and Tachinidae had the highest abundance of 10.4 and 7.2, respectively, in dolichos monocrop at flowering. Generally, at vegetative dolichos growth stage lower abundance of natural enemies was observed both in dolichos monocrop and maize-dolichos intercrop compared to flowering poddding growth stages (Table 3.11).

During the 2020 cropping season, a total of 2544 individual natural enemies were captured on farmers' fields from six (6) orders and thirteen (13) families. Parasitoids were the most abundant natural enemies with 71.3% of the total collection during this period. The order Diptera had the highest relative abundance of 65.1% and lowest in Neuroptera (1.3%). Among the families identified, the highest relative abundance was observed in Dolichopodidae (55.7%) and lowest in Coccinellidae (0.6%) (Table 3.10). In relation to the cropping system and dolichos growth stage, Dolichopodidae had the highest abundance across all growth stages for both dolichos monocrop and maize-dolichos intercrop. For instance, the highest abundance of Dolichopodidae was observed at vegetative stage in dolichos monocrop (11.1) and maize-dolichos intercrop (10.1). Carabidae was second highest in abundance after Dolichopodidae. The highest abundance for Carabidae was observed at vegetative stage in dolichos monocrop (2.9) and maize-dolichos intercrop (2.6). (Table 3.11).

Table 3. 10: Number of individuals and relative abundance of insect families collected in Njoro during 2019 and 2020 cropping seasons.

2019 cropping season

Order	Group	Families	No. of individuals	% Abundance
Hymenoptera	Parasitoid	Braconidae	92	4.2
		Ichneumonidae	74	3.3
Hemiptera	Predator	Nabidae	16	0.7
		Geocoridae	12	0.5
Diptera	Parasitoid	Tachinidae	482	21.8
		Sacrophagidae	276	12.5
		Dolichopodidae	217	9.8
		Syriphidae	683	30.9
Coleoptera	Predator	Coccinellidae	152	6.9
		Carabidae	115	5.2
		Cantharidae	59	2.7
Areneae	Predator	Salticidae	32	1.4
Total			2210	100.0

2020 cropping season

Order	Group	Families	No. of individuals	% Abundance
Hymenoptera	Parasitoids	Braconidae	136	5.3
		Ichneumonidae	21	0.8
Hemiptera	Predators	Nabidae	69	2.7
		Geocoridae	84	3.3
Diptera	Parasitoid	Tachinidae	71	2.8
		Sacrophagidae	107	4.2
		Dolichopodidae	1417	55.7
		Syriphidae	62	2.4
Neuroptera	Predator	Chrysopidae	73	2.9
Coleoptera	Predators	Coccinellidae	15	0.6
		Carabidae	317	12.5
		Cantharidae	43	1.7
Areneae	Predator	Salticidae	129	5.1
Total			2544	100

Table 3. 11: Abundance (Mean ±SE) of arthropod families collected in Njoro during 2019 and 2020 cropping seasons.

	2019 cropping season					
	Vegetative		**Flowering**		**Podding**	
NE Families	**Monocrop**	**Intercrop**	**Monocrop**	**Intercrop**	**Monocrop**	**Intercrop**
Braconidae	0.9±0.1	1.2±0.2	0.7±0.1	0.4±0.0	0.2±0.0	0.1±0.0
Ichneumonidae	0.7±0.2	1.0±0.3	0.6±0.1	0.3±0.1	0.1±0.0	0.1±0.0
Nabidae	0.1±0.0	0.1±0.0	0.0±0.0	0.1±0.0	0.2±0.0	0.0±0.0
Geocoridae	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.0	0.0±0.0
Tachinidae	1.6±0.3	1.8±0.4	7.2±1.9	3.4±1.1	3.7±0.5	2.3±0.3
Sacrophagidae	1.0±0.2	1.0±0.2	3.9±0.9	1.7±0.5	2.4±0.4	1.3±0.2
Dolichopodidae	0.7±0.1	0.8±0.1	3.1±0.7	1.3±0.4	1.8±0.3	1.0±0.1
Syriphidae	2.3±0.5	2.4±0.5	10.4±2.7	4.7±1.6	5.4±0.9	3.3±0.6
Carabidae	0.6±0.1	0.4±0.1	1.2±0.3	1.0±0.2	0.8±0.2	0.6±0.2
Coccinellidae	0.8±0.2	0.6±0.2	1.6±0.4	1.3±0.3	1.0±0.2	0.8±0.2
Cantharidae	0.3±0.1	0.2±0.0	0.6±0.1	0.5±0.1	0.4±0.0	0.3±0.0
Salticidae	0.3±0.2	0.2±0.0	0.1±0.1	0.3±0.1	0.1±0.0	0.1±0.0

Table 3.11: Continued…

	2020 Cropping season					
	Vegetative		**Flowering**		**Podding**	
NE families	**Monocrop**	**Intercrop**	**Monocrop**	**Intercrop**	**Monocrop**	**Intercrop**
Braconidae	0.6±0.3	0.5±0.2	0.3±0.1	0.8±0.4	1.4±0.4	1.2±0.4
Ichneumonidae	0.1±0.1	0.0±0.0	0.0±0.0	0.1±0.0	0.1±0.0	0.2±0.1
Nabidae	0.2±0.1	0.2±0.1	0.0±0.0	0.5±0.2	0.5±0.3	0.2±0.2
Syriphidae	0.5±0.2	0.0±0.0	0.3±0.2	0.3±0.1	0.4±0.1	0.2±0.1
Chrysopidae	0.5±0.2	1.0±0.4	0.0±0.0	0.3±0.1	0.1±0.0	0.5±0.2
Cantharidae	0.2±0.1	0.2±0.1	0.7±0.6	0.2±0.1	0.0±0.0	0.0±0.0
Geocoridae	0.0±0.0	0.0±0.0	0.2±0.1	0.8±0.4	0.8±0.6	1.1±0.6
Coccinellidae	0.0±0.0	0.0±0.0	0.1±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Salticidae	0.3±0.1	0.5±0.1	0.4±0.1	0.2±0.1	0.6±0.2	0.7±0.2
Carabidae	2.9±0.7	2.6±0.5	1.6±0.4	1.1±0.4	1.3±0.3	2.0±0.5
Sacrophagidae	1.0±0.4	1.1±0.4	0.0±0.0	0.7±0.2	0.4±0.1	0.0±0.0
Tachinidae	0.1±0.1	0.1±0.0	0.1±0.1	0.1±0.0	1.0±0.4	0.1±0.0
Dolichopodidae	11.1±1.7	10.1±1.8	7.0±1.3	8.0±2.1	6.9±1.5	5.5±1.3

3.5.4 Bean aphid natural enemies' populations as influenced by cropping season, crop growth stage and cropping system in Rongai.

During the 2019 cropping season, a total of 2532 individual natural enemies from five orders and twelve families were captured on farmers' fields. The natural enemies are classified as either predators or parasitoids. Parasitoids comprised the majority of natural enemies with 86.5% of the total collection during this period. The order Diptera had the highest relative abundance of 80.6% and lowest in Hemiptera and Areneae (1.3%). Among the families identified, the highest relative abundance was observed in Syriphidae (33.7%) and Tachinidae (23.8%). The lowest abundance was observed in Nabidae (0.6%) and Geocoridae (0.8%) (Table 3.12). In relation to the cropping system and dolichos growth stage, Syriphidae had the highest abundance of 9.0 and 7.8 at flowering for dolichos monocrop and maize-dolichos intercrop, respectively. Generally, Nabidae and Geocoridae had the lowest abundance of natural enemies both in dolichos monocrop and maize-dolichos intercrop across all dolichos growth stages (Table 3.13).

During the 2020 cropping season, a total of 2547 individual natural enemies were captured on farmer's fields from six orders and thirteen families. Parasitoids were the most abundant natural enemies with 66.7% of the total collection during this period. The order Diptera had the highest relative abundance of 57% and lowest in Neuroptera (2%). Among the families identified, the highest relative abundance was observed in Dolichopodidae (45.4%) and lowest in Cantharidae (1%) (Table 3.12). In relation to the cropping system and dolichos growth stage, Dolichopodidae had the highest abundance across all growth stages for both dolichos monocrop and maize-dolichos intercrop. For instance, the highest abundance of Dolichopodidae was observed at flowering stage in dolichos monocrop (7.3) and maize-dolichos intercrop (7). Carabidae was second highest in abundance after Dolichopodidae. The highest abundance for Carabidae was observed at vegetative stage in dolichos monocrop (3.3) and maize-dolichos intercrop (3.2) at flowering. (Table 3.13).

Table 3. 12: Number of individuals and relative abundance of insect families collected in Rongai during 2019 and 2020 cropping seasons.

2019 Cropping season				
Order	**Group**	**Families**	**No. of individuals**	**% Abundance**
Hymenoptera	Parasitoids	Braconidae	81	3.2
		Ichneumonidae	66	2.6
Hemiptera	Predators	Nabidae	19	0.8
		Geocoridae	14	0.6
Diptera	Parasitoid	Tachinidae	602	23.8
		Sacrophagidae	327	12.9
		Dolichopodidae	260	10.3
		Syriphidae	853	33.7
Coleoptera	Predator	Coccinellidae	129	5.1
		Carabidae	98	3.9
		Cantharidae	51	2.0
Areneae	Predator	Salticidae	32	1.3
Total			2532	100

2020 Cropping season				
Order	**Group**	**Families**	**No. of individuals**	**% Abundance**
Hymenoptera	Parasitoids	Braconidae	205	8.0
		Ichneumonidae	42	1.6
Hemiptera	Predators	Nabidae	158	6.2
		Geocoridae	79	3.1
Diptera	Parasitoid	Tachinidae	85	3.3
		Sacrophagidae	139	5.5
		Dolichopodidae	1156	45.4
		Syriphidae	71	2.8
Neuroptera	Predator	Chrysopidae	50	2.0
Coleoptera	Predators	Coccinellidae	42	1.6
		Carabidae	379	14.9
		Cantharidae	25	1.0
Areneae	Predator	Salticidae	116	4.6
Total			2547	100

Table 3. 13: Abundance (Mean ±SE) of arthropod families collected in Rongai during 2019 and 2020 cropping seasons.

	2019 Cropping season					
	Vegetative		Flowering		Podding	
NE Families	Monocrop	Intercrop	Monocrop	Intercrop	Monocrop	Intercrop
Braconidae	0.6±0.1	0.7±0.1	0.5±0.1	0.6±0.1	0.4±0.0	0.3±0.0
Ichneumonidae	0.5±0.2	0.6±0.2	0.4±0.2	0.5±0.1	0.3±0.2	0.2±0.1
Nabidae	0.2±0.1	0.1±0.0	0.0±0.0	0.0±0.0	0.2±0.1	0.1±0.0
Geocoridae	0.1±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.1	0.1±0.0
Tachinidae	5.8±1.9	2.5±0.6	6.3±1.9	5.6±2.0	3.5±0.7	2.2±0.4
Sacrophagidae	3.1±0.9	1.4±0.3	3.4±0.9	2.9±1.0	2.0±0.3	1.2±0.2
Dolichopodidae	2.4±0.7	1.1±0.2	2.7±0.7	2.3±0.8	1.5±0.3	1.0±0.1
Syriphidae	8.3±2.7	3.7±0.9	9.0±2.8	7.8±2.9	5.0±1.0	3.0±0.6
Carabidae	1.0±0.4	1.0±0.3	0.6±0.1	0.7±0.1	0.2±0.0	0.4±0.1
Coccinellidae	1.4±0.5	1.3±0.4	0.8±0.1	0.9±0.2	0.3±0.1	0.6±0.1
Cantharidae	0.5±0.2	0.5±0.1	0.3±0.0	0.3±0.0	0.1±0.0	0.2±0.0
Salticidae	0.0±0.0	0.2±0.1	0.2±0.1	0.4±0.2	0.0±0.0	0.3±0.1

Table 3.13: Continued…

	2020 Cropping season					
	Vegetative		**Flowering**		**Podding**	
NE families	**Monocrop**	**Intercrop**	**Monocrop**	**Intercrop**	**Monocrop**	**Intercrop**
Braconidae	1.3±0.4	0.6±0.2	1.4±0.4	1.0±0.3	1.4±0.4	1.2±0.4
Ichneumonidae	0.2±0.1	0.2±0.1	0.1±0.1	0.0±0.0	0.1±0	0.2±0.1
Nabidae	0.6±0.1	0.5±0.2	0.8±0.3	1.1±0.4	0.5±0.3	0.2±0.2
Syriphidae	0.1±0.0	0.4±0.2	0.2±0.1	0.7±0.3	0.4±0.1	0.2±0.1
Chrysopidae	0.5±0.3	0.5±0.2	0.0±0.0	0.1±0.1	0.1±0.0	0.5±0.2
Cantharidae	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.1	0.0±0.0	0.0±0.0
Geocoridae	0.1±0.0	0.2±0.1	0.2±0.1	0.2±0.1	0.8±0.6	1.1±0.6
Coccinellidae	0.2±0.1	0.1±0.0	0.3±0.1	0.2±0.1	0.0±0.0	0.0±0.0
Salticidae	0.8±0.3	0.4±0.2	0.7±0.2	0.5±0.2	0.6±0.2	0.7±0.2
Carabidae	3.5±0.7	2.6±0.6	1.9±0.6	3.2±0.9	1.3±0.3	2.0±0.5
Sacrophagidae	0.5±0.2	0.5±0.2	0.7±0.3	0.7±0.3	0.4±0.1	0.0±0.0
Tachinidae	0.3±0.1	0.3±0.1	0.4±0.1	0.5±0.2	1.0±0.4	0.1±0.0
Dolichopodidae	6.3±1.0	4.3±0.9	7.3±1.4	7.0±1.1	6.9±1.5	5.5±1.3

3.5.5 Effect of cropping systems, location and crop growth stages on bean aphids during 2019 cropping season

The results of the 2019 cropping season revealed that crop growth stage had a significant effect on bean aphid incidence, severity and abundance at the two locations and during the two cropping systems (Table 3.14). The bean aphid incidence was significantly higher at late vegetative (61.50), flowering stage (66.81) and podding stages (59.75) in Njoro location with a similar result trend observed in Rongai. The late vegetative (13.12), flowering stage (12.49) and podding stage (12.36) were severely affected in Njoro location as compared to the seedling stage where it had low severity of damage. Rongai recorded the same trend, but the severity of damage was more evident at flowering (12.10) and podding stages (13.01) (Table 3.14).

Table 3. 14: Mean aphid abundance, damage severity and percent incidence as influenced by dolichos growth stages, location and cropping system during 2019 cropping season.

Crop growth stage	**Incidence (%)**		**Severity of damage (%)**		**Abundance**	
	Njoro	**Rongai**	**Njoro**	**Rongai**	**Njoro**	**Rongai**
Seedling stage	5.00a	2.50c	1.40c	3.15c	2.50b	4.06b
Early Vegetative stage	26.18b	8.87c	5.88b	3.48c	262.19a	49.69b
Late Vegetative stage	61.50c	52.31b	13.12a	8.23b	317.50a	157.19b
Flowering stage	66.81c	58.43ab	12.49a	12.10a	291.88a	327.81a
Podding stage	59.75c	63.62a	12.36a	13.01a	353.13a	441.00a
HSD P<0.05	9.90	9.04	3.87	3.09	146.66	164.61
CV	32.03	34.51	60.67	54.87	84.74	119.13
R^2	0.87	0.88	0.59	0.61	0.54	0.49

Table 3.14: Continued…

Crop growth stage	**Monocrop**	**Intercrop**	**Monocrop**	**Intercrop**	**Monocrop**	**Intercrop**
	Incidence (%)		**Damage severity (%)**		**Aphid abundance**	
Seedling stage	5.00d	2.50b	3.31b	1.24c	3.13c	3.44b
Early Vegetative stage	23.37c	11.68b	7.37b	1.99c	213.75b	98.13ab
Late Vegetative stage	82.87b	30.93a	14.94a	6.41b	371.25b	103.44ab
Flowering stage	90.68ab	34.56a	14.79a	9.81a	530.31a	89.38ab
Podding stage	93.43a	29.93a	17.47a	7.91ab	615.00a	179.13a
HSD P<0.05	7.88	10.84	4.43	2.22	157.66	154.11
CV	18.92	20.14	24.26	27.51	21.46	30.73
R^2	0.92	0.45	0.49	0.57	0.56	0.11

Values in the same column followed by the same letter are not statistically different from each other at the 95% confidence interval using Tukey's HSD test.

The population of bean aphids also increased as the crop advanced in stage (Table 3.15). Generally, monocrop showed to have high bean aphid incidence, severity of damage and high population of bean aphids. Flowering and podding stage of monocropped dolichos showed a significantly higher bean aphid incidence but in intercropped dolichos the percent incidence was not significantly different on late vegetative, flowering and podding stages. The percent severity of damage was slightly low and ranged between 3-17% and on intercropped dolichos between 1-9%. In monocropped dolichos it was more severe at late vegetative, flowering and podding stages while in intercropped dolichos it was severe at flowering and podding stages. Higher population of bean aphids were observed at flowering and podding stage in monocropped dolichos while in intercropped dolichos the abundance of bean aphids was at podding stage (Table 3.15).

3.5.6 Effect of cropping systems, location, and crop growth stages on bean aphids during the 2020 cropping season

Data on seedling stage was not taken during the 2020 cropping season. The aphid incidences ranged from 15-21% and 6-24% in Njoro and Rongai, respectively. Early vegetative stage and podding stages in Njoro location recorded a low percent bean aphid incidence while in Rongai the early vegetative recorded a low percent incidence which was significantly different from the other crop stages. Njoro recorded a more severe damage caused by bean aphids which ranged from 5 to 10% as compared to Rongai from 2 to 9%. Low severity was observed at late vegetative stage (5.82) in Njoro location while Rongai was early (2.24) and late vegetative stages (4.99). As the crop advanced on stage the population of bean aphids lowered in both Njoro and Rongai locations. The percent aphid incidence in monocrop was significantly lower at early vegetative stage while there was no significant difference in intercropped dolichos. The bean aphid severity was less at flowering stage in both monocrop and intercrop systems. Additionally, the bean aphid aabundance showed no significant effect across the crop growth stages in either monocropped or intercropped dolichos (Table 3.16).

Table 3. 15: Mean aphid abundance, damage severity and percent incidence as influenced by dolichos growth stages, location and cropping system during 2020 cropping season.

Crop growth stage	**Incidence (%)**		**Severity of damage (%)**		**Abundance**	
	Njoro	**Rongai**	**Njoro**	**Rongai**	**Njoro**	**Rongai**
Seedling stage	-	-	-	-	-	-
Early Vegetative stage	15.81b	6.18b	10.25ab	2.24b	30.00ab	8.44b
Late Vegetative stage	26.75ab	20.81a	5.82c	4.99b	27.50ab	34.69ab
Flowering stage	33.18a	21.56a	12.44a	9.16a	59.69a	27.81ab
Podding stage	21.93b	24.62a	6.56bc	8.20a	22.19b	111.25a
HSD P<0.05	11.04	12.89	4.19	2.79	32.45	90.79
CV	63.82	99.51	67.53	64.23	131.49	281.44
R^2	0.25	0.27	0.31	0.43	0.21	0.13

	Monocrop	Intercrop	Monocrop	Intercrop	Monocrop	Intercrop
Seedling stage	-	-	-	-	-	-
Early Vegetative stage	11.62b	10.37a	5.87b	6.62a	19.69a	18.75a
Late Vegetative stage	29.12a	18.43a	6.89b	3.91b	48.75a	13.44a
Flowering stage	36.12a	18.62a	14.53a	7.07a	60.31a	27.19a
Podding stage	31.68a	14.87a	9.20b	5.56ab	88.75a	44.69a
HSD P<0.05	14.35	9.05	4.26	2.69	85.84	43.88
CV	74.68	82.10	65.97	65.59	222.91	238.16
R^2	0.22	0.19	0.34	0.32	0.13	0.07

Means in a column followed by the same letters are not significantly different at $P<0.05$ using Tukey's HSD test.

3.5.7 Effect of cropping system, season, and location on abundance of parasitoids

In the year 2019 all the parasitoids that emerged from *Aphis fabae* infested sentinel plants were DNA barcoded and identified (Table 3.16). All the primary parasitoids were *Aphidius* spp., with *Aphidius colemani* being the only species identified to species level. Aphidius species were the most abundant 129 of which *Aphidius colemani* comprised the majority at 117. The species can also be grouped together with *A. colemani* in a phylogenetic tree. Other parasitoids identified included *Asaphes* spp., *Phaenoglyphis* spp., *Phaenoglyphis villosa* and *Pteromalidae* spp.

Table 3. 16: Number of emerged parasitoids from *A. fabae* sentinel bean plants during the 2019 season.

Identity of specimens	**Count**
Aphidius	129
Aphidius colemani	117
Asaphes	3
Pachyneuron	3
Phaenoglyphis	7
Phaenoglyphis villosa	3
Pteromalidae	26
NA	23

NA represents where sequencing was unsuccessful, either because sequencing was unsuccessful or there was no match with >90% similarity.

In 2020 all barcoded parasitoids were primary parasitoids that included *Aphidius* sp., *Aphidius colemani*, *Lysiphlebus fabarum* and *Lysiphlebus* sp (Figure 3.3). Analysis by GLM showed that there were significantly ($P<0.001$) more parasitoids in Njoro than Rongai (Figure 3.4). Hence the location in which the sentinel plants were deployed was found to have a significant effect on the number of emerging parasitoids. Results showed that there was no correlation between Simpson's diversity index and number of parasitoids (r=-0.049, P=0.79; Figure 3.5) or between species richness and the number of parasitoids (r=0.024, P=0.9; Figure 3.6).

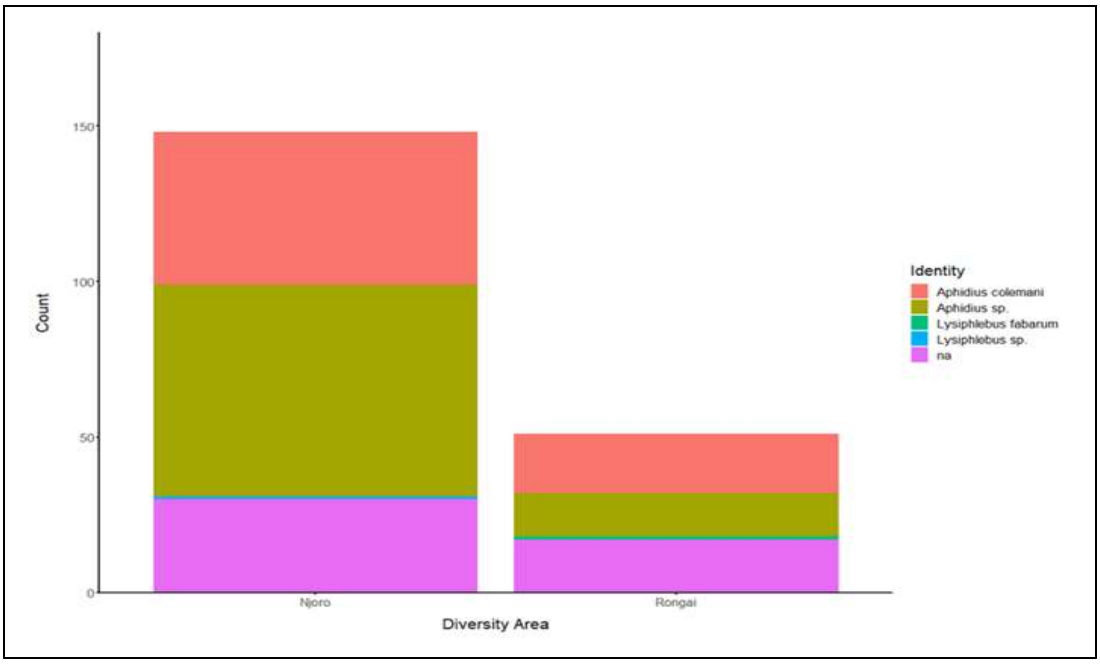

Figure 3. 3: The number of parasitoids identified from *A. fabae* on sentinel plants during the year 2020.

NA represents where sequencing was unsuccessful, either because sequencing was unsuccessful or there was no match with >90% similarity.

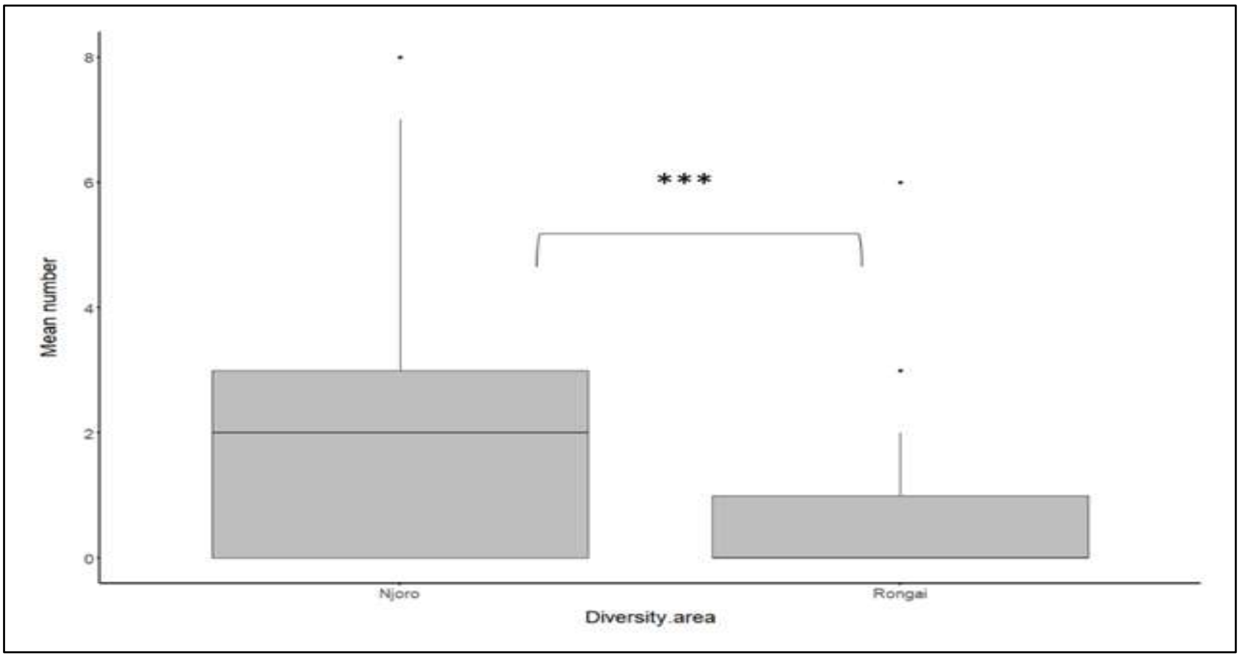

Figure 3. 4: The mean number of parasitoids emerged from sentinel plants in Njoro and Rongai.

Significant differences are indicated by * and were calculated in R-Studio using a GLM.

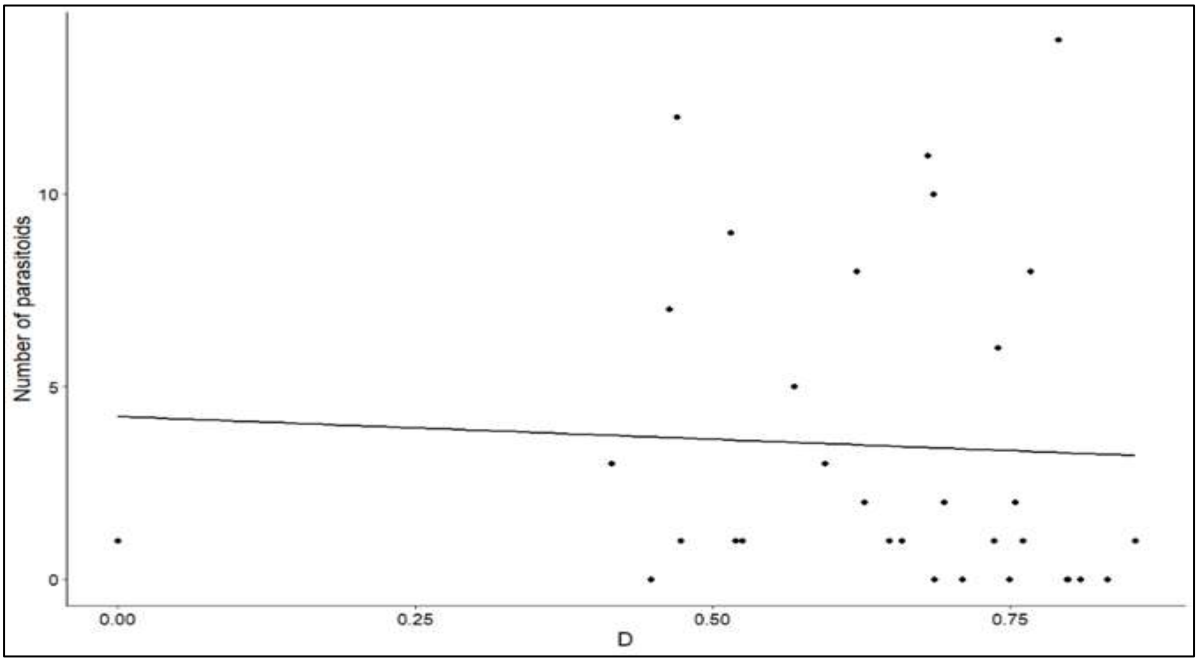

Figure 3. 5: The relationship between the number of parasitoids emerged from sentinel plants and the Simpson's diversity index (D) of the field margin plants surrounding each field.

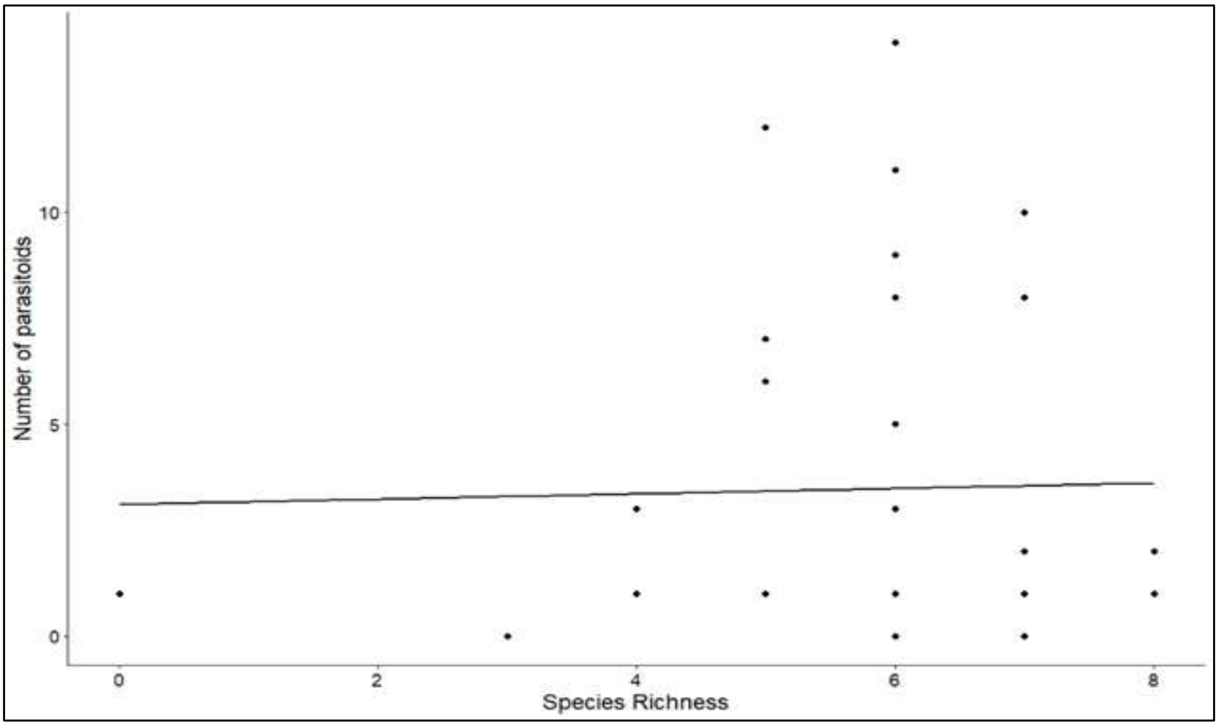

Figure 3. 6: The relationship between the number of parasitoids emerged from sentinel plants and the species richness of the field margin plants surrounding each field.

3.5.8 Dolichos grain yield and intercropping productivity

Results showed no significant differences between the two locations and cropping systems on 100 seed weight. Higher grain yield was observed in monocropped dolichos as compared to intercropped dolichos in all the cropping seasons. Across the two cropping seasons Rongai had a higher land equivalent ratio 1.29 ,1.24 for 2019 and 2020, respectively compared to Njoro with 1.24, 0.80 for 2019 and 2020, respectively. (Table 3.17).

Table 3. 17: Mean 100 seed weight, aboveground biomass, grain yield and land equivalent ratio as influenced by location and cropping system of dolichos.

Location/Cropping system	100 seed weight (g)	Biomass (ton ha^{-1})	Yield (ton ha^{-1})	Land equivalent ratio (LER)
	2019 cropping season			
Njoro	25.02a	-	1.6a	1.24^{b}
Rongai	25.62a	-	1.7a	1.29^{a}
HSD P<0.05	0.82	-	1.2	0.06
Monocrop	25.02a	-	2.8a	
Intercrop	25.42a	-	0.5b	
HSD P<0.05	0.72	-	1.0	
	2020 cropping season			
Njoro	23.9a	3.7a	1.7a	0.80^{b}
Rongai	23.8a	2.5b	0.7b	1.24^{a}
HSD P<0.05	2.7	9.3	0.2	0.03
Monocrop	22.9a	4.3a	2.0a	
Intercrop	24.8a	1.7b	0.4b	
HSD P<0.05	2.69	9.3	1.4	

Values in a column followed by the same letter are not significantly different using Tukey's HSD test at P<0.05.

3.6 Discussion

Conservation biological control practices include habitat manipulations that provide non pest resources and selectively enhance natural enemies' effectiveness. Field margin vegetation is important and serves many functions including serving as habitats for an array of natural enemy species (predators and parasitoids) and protecting the crop from pesticides or fertiliser drift (Holland *et al*., 2016). Njoro had an abundant field margin vegetation of annual and perennial plant composition compared to Rongai location. This difference might be as a result of varying farm management practices employed in the two locations and also the climatic conditions prevailing in the two locations; these affect the species composition (Appendix A). Njoro farms constitute regularly disturbed environments while in Rongai most farmers do graze animals as an agricultural practice. Natural field margin vegetation diversity may have a suppressive effect on pest abundance through bottom-up mechanisms that disrupt the pest's ability to locate or access the host plant (Balzan *et al*., 2016). Vegetation complexity, in more species diverse field margins, can mediate multi predator effects on herbivores by providing microhabitats that act as a refuge. Findings from this study have demonstrated that field margins around farmlands can provide flowering resources and alternate prey, necessary for enhancing natural enemies' diversity and reducing aphid infestation level.

Cynodon dactylon was recorded as the most abundant perennial field margin vegetation species in Rongai. The grasses in Rongai location establish faster and have a dense cover preventing other field margin species from establishing. Carvell *et al.* (2007) reported that field margins that are composed of perennials can increase arthropod diversity and provide nectar and pollen food sources for a long period of time. The current study results are in accordance with observations of Critchley *et al*. (2006) who reported that grasses establish better in locations with minimal disturbance. The blackjack *(Bidens Pilosa)* being the most abundant annual species has the ability to establish quickly due to its capacity to produce many seeds per crop and its characteristic trait to achieve a wider dispersal (Shimamoto *et al*., 2011).

The effect of location on species composition could also be explained by the different soil types and properties which varied across the two locations (Appendix B). The 'natural enemies hypothesis' states that increase in abundance of arthropod predators and parasitoids in species rich field margin vegetation results in the regulation of agricultural pests to a lower level in diverse vegetation than in pure stands (Root, 1973). Hence, high abundance of arthropod population in Rongai location for the two cropping seasons. These results are in agreement with Letourneau *et al*. (2011) who reported that field manipulation provided insight about the underlying mechanisms that lead to the positive relationship with herbivores and their natural enemies.

The study of insect variety is crucial for understanding biodiversity, community dynamics, and global change (Wagner *et al.*, 2021). The diversity of insects collected from pan traps, sweep nets and sticky traps in the two locations over the two seasons differed in their indices, abundance, richness and evenness. The traps were proposed to collect as follows; crawling insects by pan traps, flying insects outside the field margins by sweep nets and flying natural enemies inside the crop by sticky traps. Additionally, it is scientifically impossible to collect all species families with one type of trap because different insects have different locomotive traits and cues in the search for food, mate, and niche (Page & Bernal, 2020). Sweep netting, pan trapping and use of sticky traps are common methods in assessing the population dynamics of arthropods. The arthropods are important in the ecosystem as most play a role in being the natural enemies of agricultural pests (Spafford & Lortie, 2013). Sweep netting and pan trapping are associated with low lying flowering vegetation in a wide variety of habitats.

In this study, the seedling stage recorded a rich insect abundance that differed significantly with Lingaraju and Biradar (2015) in pigeon pea who reported that natural enemies became more abundant as the crop advanced in age but reduced at the reproductive stage. The extent of damage to crop plants from pest insects depends on the foraging behaviour of the insect's feeding stage. Pest species depend on the pest's ability to colonise the field area. Colonisation ability, in turn, is related to pest density and to individual propensity to oviposit or feed on multiple plants within a field. This explains why the bean aphids were able to locate and colonise dolichos crop at seedling stage and be more abundant. The difference could also be attributed to the fact that most of the field margin vegetation was at flowering stage hence encouraging the high population of natural enemies.

Among the insect families, Formicidae was the most abundant. Although they are not the natural enemies of bean aphids, they play an important role in protecting the bean aphids from other predators and in turn they benefit from harvesting honeydew (Rice & Eubanks, 2013). The identified families that constituted bean aphid natural enemies were Braconidae, Ichneumonidae, Nabidae, Geocoridae, Tachinidae, Sarcophagidae, Dolichopodidae, Syriphidae, Chrysopidae, Coccinellidae and Carabidae which were grouped into predators and parasitoids. Similar study observations were made by Ohnesorg *et al.* (2009) who reported species in the Braconidae, Ichneumonidae and Coccinellidae families as natural enemies of bean aphids in soya bean. Studies by Dale *et al*. (2020) suggested that presence of flowers is likely to be beneficial to several families of arthropods and natural enemies. Most hymenopteran parasitoids and predators, have short mouthparts and get access to energy requirements by feeding on relatively accessible sugar sources, such as exposed floral and extrafloral nectar from the field margin vegetation (Winkler *et al*., 2009). The most abundant natural parasitoids were the Syriphidae and Tachnidae and their population are associated with the abundance of flowering field margin vegetation. The adult stage of Syriphidae and Tachnidae totally depends on pollen and nectar from the field margin vegetation while the larval stages feed on the aphids (Amaral *et al*., 2013). Dolichopodidae and Coccinellidae are predators which were among the abundant species whose adults similarly depend on the nectar from field margin vegetation.

The results showed that bean aphids are among the most serious and widespread insect pests attacking lablab beans. Dolichos monocrop and intercrop had different bean aphid densities and natural enemies' abundance. Cropping system influences the abundance, diversity and relative importance of pests, natural enemies and yield of crops (Garratt *et al*., 2011; Mwani *et al.,* 2021; Rusch *et al*., 2010). The pest populations were lower in intercropped dolichos as compared to monocrop dolichos which might be due to low concentration of food resources for pests, or increased abundance or effectiveness of natural enemies in the intercrop. These results are in agreement with Srinivasa *et al.* (2012) who reported that the intercropping castor plants and the legumes recorded lower population levels of *A. janata* as compared to the monocropped castor. In regard to cropping systems intercropping has been reported as a potential solution to maximise biodiversity and abundance of arthropods (Brandmeier *et al*., 2021). Additionally maize could have caused a shading effect to the dolichos bean hence the lower population of bean aphids on intercropped dolichos. These results concur with Zou *et al*. (2022) who reported that maize shading on tea inhibited the occurrence of major pests in tea gardens, which was conducive to enhancing the biodiversity of tea gardens. Shading results in reduction of solar radiation that reaches the dolichos crop hence creating microclimate with varying intensity of sunlight that influences the build-up of the bean aphid population (Hill *et al*., 2010).

Crop growth stage has a significant effect on bean aphid incidence, severity and abundance. The bean aphid population distribution and severity of damage depends on dolichos crop growth stages. The population of bean aphids were generally low at early growth stages and increased as the crop progressed, which was consistent with the findings of Abudulai *et al.* (2012). This high reproductive rate was probably due to more suitable temperatures and relative humidity that favour insect multiplication as the season progressed or increase in food sources by the growth of new leaves. Taylor (1962) reported that *Aphis fabae* populations are usually denser and confined to the edges of bean crops, particularly on the windward sides hence the development of new leaves is always prone to aphid damage. Dolichos seedling stage recorded a low population of bean aphids since the pest had not yet located its host at that stage. At the podding, stage the severity of damage was higher in the monocrop cropping system as compared to intercropped dolichos. The differences in severity of damage between the two cropping systems may be due to presence of non-host plants i.e maize that reduced the host finding ability. These results agree with Chabi-O *et al.* (2005) who reported that intercropping maize with non-host plants substantially reduces stem borer infestation of maize in southern Cameroon although their larval densities were higher during the vegetative stages than the reproductive stages of maize.

Most existing management strategies have been based on the indiscriminate use of pesticides in agriculture. However, results from this study showed that the bean aphid population can be managed to levels that don't reach the economic injury levels of dolichos beans. The levels of natural enemies were different between the two cropping seasons mainly due to seasonal variations in climatic conditions (Appendix 1). Temperature and humidity are some of the factors that affect the life expectancy and reproduction of insects (Khaliq *et al*., 2014). However not all natural enemies were abundant in the intercrop system since intercropping maize and dolichos may have altered microhabitat conditions (i.e soil moisture, temperature and light penetration through the canopy), making the environment less suitable for some species. The predators of bean aphids began to appear from the earliest growth stages of dolichos. The most dominant natural enemy being the predatory flies which was captured by the pan traps and the sweep net while the major families of the parasitoids identified were the hymenopterans. Parasitoids use semiochemical and physical stimuli to locate and recognize their hosts. These responses are either due to aphid sex pheromones acting as kairomones, or due to aphid-induced plant volatiles, acting as synomones (Rehman & Powell, 2010). Abundant populations of invertebrate taxa in the monocrop system indicated that the natural enemies were attracted to the released aphid pheromones. High populations of the natural enemies could also have been attributed to the field margin vegetation that was surrounding the two cropping systems which provided pollen and nectar food sources. The adult predators and parasitoids are known to explore other food sources such as nectar and pollen (Landis *et al.,* 2005). The predatory spiders were abundant at dolichos seedling stage because whenever they locate the bean aphids, they start adapting by creating orb-webs near its host. Results from the sentinel plants revealed a high population of parasitoids in Njoro location. This might be due to the fact that this location had a high population of the bean aphids as the source of food.

Aphidius colemani parasitoid is thought to have given natural aphid population management just as the predators. It has been documented to be responsible for natural aphid pest regulation across a variety of crops. It can maintain aphid populations at levels similar to those resulting from pesticide applications. Other studies have shown that *Aphidius colemani* is a widely known parasitoid with many positive attributes capable of controlling more than 41 host species of the aphid. This makes it one among the first biological control agents that have been commercialised for growers in many countries. In ideal circumstances, *A. colemani* can maintain aphid populations at levels similar to those resulting from pesticide applications (Vásquez *et al*., 2006). *A colemani* has greater dispersal distance and searching (Heinz, 1994). When used in conjunction with the predatory dipteran *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) in a greenhouse trial, the majority of the aphid control was attributed to *A. colemani* (Talebi *et al*., 2006). Compared to three others economically important Aphidiinae parasitoids (including *Aphidius matricariae*), *Aphididius colemani* was the most effective at controlling *Aphis gossypii* in the greenhouse due to its higher parasitism rate on this host (Van Steenis, 1995). *Aphidius colemani* is also relatively easy to rear commercially, making it one of the more cost-effective aphid biological control agents on the market. The findings from this study is therefore a prerequisite for a practical step of building a database of parasitoids and natural enemies in Kenya to be used for insect pest management.

Regarding the cropping systems, dolichos monocrop had a higher grain yield. In similar studies by Belay *et al*. (2009); Seran and Brintha (2010); Tadesse *et al.* (2012) show that intercropped systems are more profitable through economic analysis and are better in pest population reduction hence high yields and economic returns. Rongai had a higher land equivalent ratio (LER) value compared to Njoro across the two cropping seasons. Higher LER value of 1.29 in Rongai for 2019 indicate that 28% (0.28 ha) more land area would be needed by monocropping to equal productivity of intercropping. Similarly in 2020, a LER value of 1.24 was observed in Rongai indicating that 28% (0.28 ha) more land area would be needed by monocropping to equal productivity of intercropping. Intercropping maize and dolichos led to a significant reduction in dolichos yield. This observation is attributed to physiological aspects such as reduced stomatal conductance and photosynthetic rate. In addition to insect pest infestation, crop yield is influenced by photoassimilates synthesis and partitioning by plants

(Mwamlima *et al*., 2022). Generally, the yield aspect is influenced by many factors such as plant competition for resources such as water and light and type of intercropping. However, there was no significant correlation of bean aphid reduction and yield increase in both monocrop and intercropped dolichos. Studies by Letourneau *et al.* (2011) showed that pest reduction does not directly translate to yields under field conditions. Due to the study limit, the research was limited and did not obtain enough data to address the matter.

3.7 Conclusion

The results of this study have demonstrated that intercropping and field margin vegetation can provide suitable habitat for invertebrates and other fauna. *Cynodon dactylon, Chloris gayana and Parthenium hysterophorus* are grass species that occur with high abundance in Rongai location. Grass species tend to establish faster in environments where there is little soil disturbance such as the case in Rongai location. Syriphidae family was richer in both locations over the two seasons making it a promising natural enemy of bean aphids. Predators and parasitoids population can be enhanced by use of field margin vegetation and integration of diverse cropping systems to conserve the natural enemies. Thus, the present study has shown that aphid parasitoids can play an important role in suppressing bean aphid populations. *Aphidius colemani* was found to be the most abundant parasitoid in the farmlands within the locations where the studies were conducted. Hence it can be concluded that it can be greatly deployed in the management of bean aphids alongside other natural enemies such as predators.

CHAPTER FOUR

INFLUENCE OF PLANTED FIELD MARGIN VEGETATION SPECIES AND CROPPING SYSTEM ON BEAN APHID NATURAL ENEMIES IN REDUCING APHID DAMAGE IN DOLICHOS (*Lablab purpureus*)

Abstract

Field margin plants surrounding crops are postulated to boost natural enemy populations for biological pest control. Field and cage experiments were carried out to determine the effect of planted field margin vegetation and cropping pattern in supporting natural enemies of bean aphids (*Aphis fabae*) on dolichos, an orphan crop legume. The experimental treatments were comprised of maize-dolichos intercrop and dolichos monocrop either with or without planted field margin vegetation comprising four plant species (*Chenopodium album*, *Bidens pilosa*, *Galinsoga parviflora* and *Tagetes minuta*), around the plots. The plots measured 10m x 10m, 20m apart and the treatments were arranged in a RCBD with four replicates. A cage experiment was conducted to determine the extent to which the planted field margin vegetation supported the parasitoid wasp, *Aphidius colemani*, to parasitize bean aphids. A total of 2029 insects from 10 families were collected using sticky cards and pan traps. In comparison to plots with no field margin vegetation, the presence of plant-rich field margins vegetation increased the abundance of natural enemies by 9.5% and supported higher populations of Braconidae (parasitoids) and damsel bugs (predators). The maize-dolichos intercrop had 15.5% lower abundance of natural enemies than the dolichos monocrop. Higher grain yield was observed in plots with a field margin vegetation (300 kg ha^{-1}) compared to plots without field margin vegetation (210 kg ha^{-1}) in both seasons. The presence of a companion plant did not significantly affect the performance of parasitoids. In relation to supporting aphid parasitization, *Bidens pilosa* was connected with the highest number of mummies (8.28). The lowest number of mummies were observed in cages with *Tagetes minuta* (3.44). These findings highlight the potential of plant-rich field margins to increase the number of natural enemies and improve aphid conservation biocontrol.

4.1 Introduction

Increased agricultural productivity is key in accelerating global food and nutrition security (Steensland & Thompson, 2019). The continued agricultural intensification and increased food production has however resulted in the loss of natural habitat and biodiversity. On the other hand, crop losses brought on by insect pests have been worsened by climate change, with far-reaching effects on food security and crop productivity. (Foley *et al*., 2005). The natural enemies of pests that seek refuge in non-crop habitats are disrupted by agricultural systems that heavily depend on automation and synthetic inputs like pesticides. (Balzan *et al.,* 2016; Bommarco *et al*., 2013). This necessitates strategies that restore biocontrol services by strengthening the role of natural enemies in pest management while weighing the advantages of agricultural expansion. Safeguarding significant biological control agents offers a sustainable approach to pest management, potentially lowering reliance on the use of synthetic pesticides (Ochieng *et al*., 2022). Non-crop habitats offer a refuge for invertebrate species and a chance for natural enemies to move between crop and non-crop areas. (Balmer *et al*., 2013; Morandin *et al*., 2014; Tscharntke *et al*., 2005). The loss of these habitats leads to low-diversity, simplified landscapes that change the invertebrate community in the ecosystems, which in turn affects how well natural pest management works (Bianchi *et al*., 2006). Therefore, there is an urgent need for effective interventions to preserve the variety and quantity of natural enemies in agricultural ecosystems and boost food production in ways that are environmentally friendly. (Amoabeng *et al*., 2020; Ratnadass *et al*., 2012).

Ecological intensification techniques include planting of flowering field margins and intercropping that supply resources such as pollen and nectar and so can be connected with increased fecundity and longevity of natural enemies (Jacob & Evans, 2000). In particular, parasitoid wasps (e.g. *Aphidius colemani*) a significant natural enemy in East Africa (Woolley *et al*., 2022) are essential in the control of many insect pests. It has been demonstrated that these parasitoids benefit from obtaining access to nectar sources and alternate hosts (Gurr *et al*., 2017; Kishinevsky *et al*., 2017; Souza *et al*., 2019). The diversity and number of natural enemies rely on non-crop plants to provide them with food, shelter, diapause habitats, and prey (Alignier *et al*., 2014; Langellotto & Denno, 2004; Zhao *et al*., 2021).

Dolichos bean (*Lablab purpureus* (L.) Sweet) is (Minde *et al*., 2021) is an underrated orphan crop legume with significant potential to reduce risks to global food security brought on by climate change. Dolichos production is however inhibited by insect pests, particularly bean aphid (*Aphis fabae* Scopoli) which can result to 40-90% yield loss (Abate & Ampofo, 2003; Nahashonon *et al*., 2016). Little research has been conducted on integrated pest control, which includes the function of field margin plants in promoting natural pest regulation in orphan legume crops such as dolichos. The main objective of this study was to determine the pest management potential of natural enemies through ecological intensification of field margins. Various farming strategies and kinds of field margin plants can enhance yields and help natural pest control. It was proposed that intercropping, field margin vegetation, and alternative hosts for natural enemies could lower insect occurrence in agricultural fields. Major weed species that are frequently found in farmlands were examined to see if they supported the diversity and abundance of a natural enemy of bean aphids in dolichos. The study also focused on approaches to enhance natural enemy population using manipulated field margin vegetation in different cropping systems such as intercropping to manage bean aphids in dolichos. The results from this study are aimed at providing information to farmers on how to implement a sustainable IPM option for the management of bean aphids on dolichos that can be extended to other staple food crops.

4.2 Materials and methods

4.2.1 Study Site

The study was carried out at the Agronomy Field Station, Egerton University, Nakuru County, Kenya. The site is 2200 meters above sea level and is located at 0° 19' 0"S and 36° 06' 0" E. The temperature ranges from 17°C to 22°C, while the annual rainfall ranges from 1000 to 1250 mm. The soils fall under the category of vintric mollic andosols (Jaetzold *et al*., 2012).

4.2.2 Field experiment

This experiment was designed to determine the influence of planted margin species and cropping systems on the abundance and diversity of bean aphid natural enemies.The experiment was conducted from March to June 2020 during the long rains. The experimental treatment consisted of 2 factors where the first treatment factor was field margin vegetation consisting of four weed species. The field margin plant species were carefully chosen based on their relative abundance in the ecosystem which included: *Chenopodium album* (Amaranthaceae), *Bidens pilosa* (Asteraceae), *Galinsoga parviflora* (Asteraceae) and *Tagetes minuta* (Asteraceae). In addition, the selection of these plant species was based on earlier studies which designated their role in supporting beneficial insects (Ditner *et al*., 2013; Ndakidemi *et al*., 2021). The plant species were sown as seed mixtures two weeks before planting the lablab crop. The plots which had no field margin vegetation were left bare throughout the dolichos growth cycle. The second treatment factor was the cropping system where dolichos were either planted as monocrop or intercrop (with maize). Dolichos monocrop and the dolichos-maize intercrop were planted either in the presence or absence of field margin vegetation. The experimental plots measuring 10 m x 10 m, 20 m apart were demarcated. The experimental treatments were laid out in a randomized complete block design (RCBD) with four replications in a 2x2 factorial arrangement (Figure 4.1). Dolichos monocrop was planted using a spacing of 0.5 m × 0.3 m. In the maize-lablab intercrop, maize was planted at a spacing of 0.75 m × 0.3 m, 1 seed per hill and dolichos was planted between the maize rows at an intra-row spacing of 0.15 m, 2 seeds per hill. Chemical pesticides were not applied on the crops during the season to reduce mortality of insect pests and natural enemies due to chemicals. The planted field margin plants were left to grow up to the flowering stage and to coincide with the vegetative stage of the dolichos crop.

Movement of insects was assessed from the field margin plants into the crop on the plots by applying four different fluorescent powders (pink, yellow, green and blue) to the flowers of the planted margin species. The application fluorescent dyes followed procedures from previous similar studies reported by (Mkenda *et al*., 2019; Wratten *et al*., 2003). Insects visiting treated flowers would passively pick up the dye, which could then be used to ascertain if those insects captured in the crop had previously visited field margin flowers. The dyes were prepared according to the manufacturer's instructions; mixing 100 g of powder with one litre of water to make a thick paste that was applied to all open flowers with a stiff medium hard brush. The different dyes were assigned to the different plant species as follows; yellow for *Chenopodium album*, green for *Bidens pilosa*, pink for *Galinsonga parviflora* and blue for *Tagetes minuta*. Transparent Perspex sticky traps (Plaskolite Inc, Columbus, OH, USA) measuring 10 cm × 25 cm and coated on both sides with Tanglefoot (The Tanglefoot Company Grand Rapids, MI, USA) were placed in each experimental plot at a distance of 2 m and 4 m from the field margin. The sticky traps were suspended by a string at 30 cm above the lablab crop canopy to capture flying natural enemies. Similarly, to capture crawling natural enemies, pan traps made of transparent round plastic containers measuring 20 cm diameter by 5 cm depth filled with a premixed liquid solution containing 250 ml of water, 5 g of salt to preserve the natural enemies and 5 ml of odourless liquid detergent to break the surface tension, were placed at ground level just below the sticky traps and left in the field for 48 h. The choice of transparent traps was chosen to remove bias for colour spectra influences on insect movement.

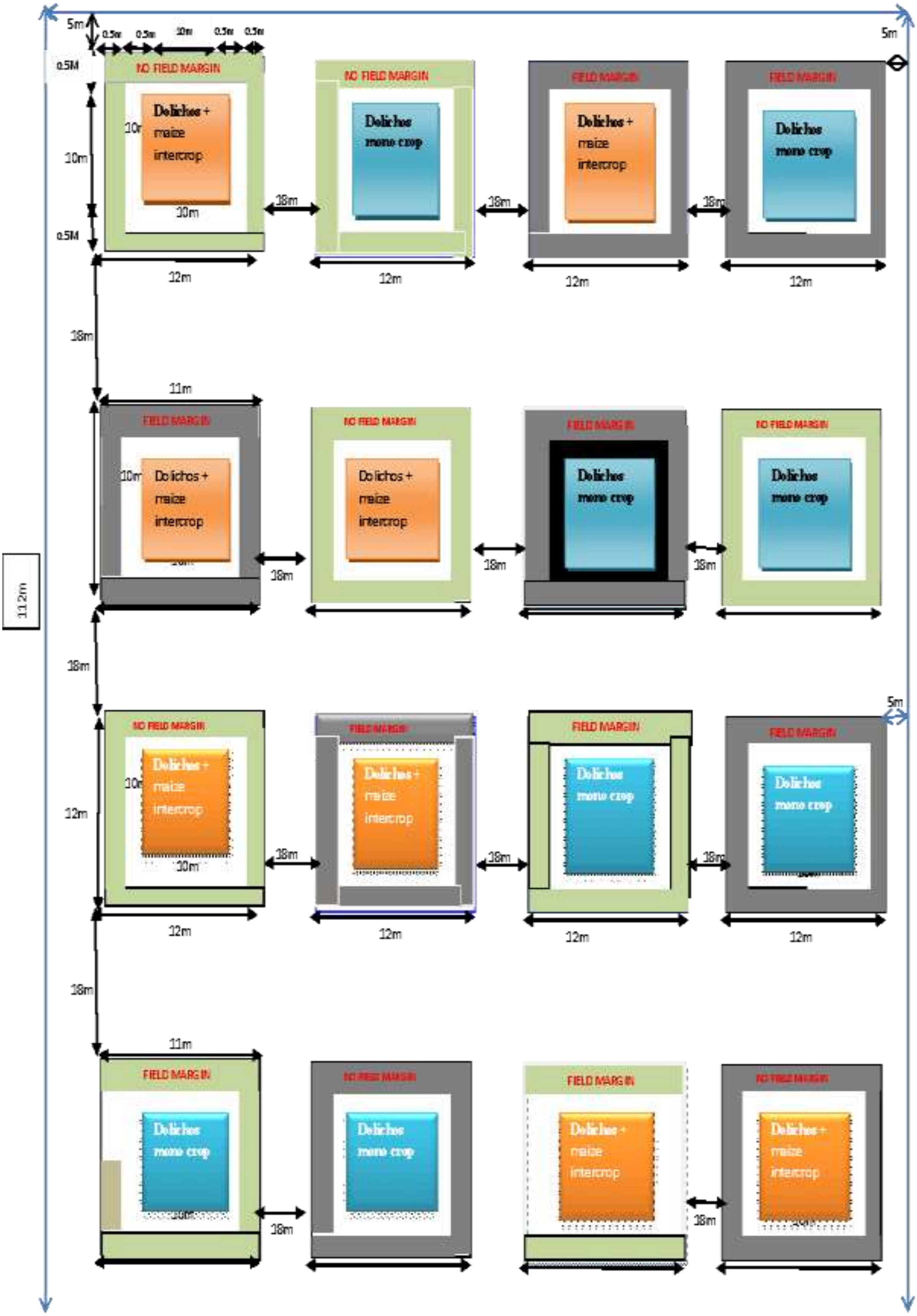

Figure 4. 1: Field experimental layout for on-station trial.

Aphid parasitization was assessed using sentinel plants, which were potted lablab seedlings infested with 60 bean aphids (*A. fabae*) each. Sentinel plants were placed in the same experimental plots immediately (same day) after removal of transparent sticky traps and pan traps (48 hours (h) after the fluorescent dye application). Every plot (lablab monocrop or maize-lablab intercrop) received two sentinel plants: one placed at the centre of the crop (5 m from the outermost row) and at the centre of margin vegetation (0.5 m from the outermost row). The plants were left in the field plots for 7 days to allow aphid parasitoids to lay eggs.

To verify results from the field experiment on the potential of individual margin species performance to support enhanced aphid natural enemy (parasitoids) abundance and bean aphid suppression, a cage experiment was conducted during the September–November, 2020 seasons. The cage experiment was laid out in an open field 4 km away from the crop fields, arranged in RCBD with four replicates. Each of the four replicates had six metallic frame cages measuring 1 m × 1 m × 2 m (length x width x height) set 2 m apart giving a total of 24 cages. Each cage was covered with white fine net (0.2 mm mesh diameter) with two zips on one side for ease of access (Plate 4.1). A cage contained either one of the four field margin plant species (*C. album*, *B. pilosa*, *G. parviflora* and *T. minuta*) (Plate 4.1) in a 20 L planting pot and retained up to flowering stage or was a control without a plant, all plant-containing cages was also provided a water source for parasitoids. Control (positive and negative, respectively) cages contained either 10% sucrose solution only or only water with no companion plant; one negative and one positive control was placed in each plot. The sucrose and water were supplied using 50 mL glass bottles with wicks. A sentinel plant infested with 80 bean aphids (*A. fabae*) from a laboratory culture was then introduced in each cage. A total six, 2-day old *A. colemani* parasitoids were released into the cages at a ratio of two males to four females and allowed to colonize freely for 30 days. *A. colemani* was selected because it was the most abundant primary parasitoid identified to control bean aphids in lablab from another experiment in the same study system (Mkenda *et al.*, 2019).

Plate 4. 1: Exclusion cages (A) and individual margin plant species (B).

4.3 Data collection

4.3.1 Diversity, abundance and temporal distribution of aphid natural enemies

To determine enhanced support of individual margin species plants to aphid natural enemies fluorescent dyes were employed to trace margin visitation by natural enemies and their subsequent return to the crop. Insect movement from field edge vegetation into experimental plots was tracked by putting four different fluorescent dyes (pink, yellow, green, and blue) (Plate 4.2) to the flowers of the planted margin species (Karp *et al*., 2018; Mkenda *et al*., 2019; Wratten *et al*., 2003). One dye was assigned to a different plant species as follows; yellow for *C. album*, green for *B. pilosa*, pink for *G. parviflora* and blue for *T. minuta* (Plate 4.2). Insects visiting flowers would passively pick up the dye, making it easy to determine whether insects trapped on the crop visited the field margin flowers. Using a stiff, medium-hard brush, the thick paste produced by combining 100 g of powder with 1 L of water to create the dyes was applied to all of the open flowers on the plot in accordance with the manufacturer's instructions. Each plot had four transparent Perspex sticky traps (Plaskolite Inc.) that were 10 cm by 25 cm and had Tanglefoot (The Tanglefoot Company) coated on both sides. Each trap was placed facing the opposite direction of the plot at a distance of 5 metres from the field margin. To capture the flying natural enemies, the sticky traps were hung 30 cm above the lablab crop canopy with a string.

The choice of transparent traps was selected to reduce bias for colour influences on insect movement and prevent artificially inducing movement within the crop. Additionally, to capture the crawling natural enemies, transparent circular plastic pan traps with a diameter of 20 cm and a depth of 5 cm were positioned at ground level directly beneath the sticky traps. These traps were then left in the field for 48 hours, filled with a premixed liquid solution that contained 250 mL of water, 5 g of salt to preserve the natural enemies, and 5 mL of odourless liquid detergent to break the surface tension. Early vegetative, late vegetative and podding growth stages were the three lablab crop stages at which traps were used. All flowers were dyed right before the trap was positioned. Insects on sticky traps were taken to the laboratory so as to identify them. After sieving and cleaning the pan-trapped insects with clean water, they were transferred using a camel hair brush with a size of 1/8 inch in width and 1/2 inch in length to 50 millilitre plastic falcon tubes that were filled with 25 millilitres of 70% ethanol. Using an illuminated dissecting microscope in a dark environment, the collected insect bodies were examined for signs of the various dyes. Only natural enemies that had traces of dye on their body were recorded as having visited or been supported by the planted margin species. Using taxonomy keys, all of the obtained specimens regardless of the presence of dye were numbered and identified down to the family level using a dissecting microscope (Leica ZOOM 2000 Inc.) at a 200× magnification (Sadof *et al*., 2014).

Plate 4. 2: Different fluorescent dyes (A) and application of the fluorescent dyes to margin plants (B).

4.3.2 Bean aphid parasitization in the lablab cropping system and field margin vegetation

Sentinel plants were used to assess aphid parasitization which consisted of potted dolichos seedlings infested with 60 bean aphids (*A. fabae*) each (Plate 4.3). Sentinel plants were placed in the equivalent experimental plots immediately (same day) after removing the transparent sticky traps and pan traps (48 h after the fluorescent dye was applied). Two sentinel plants were planted in each plot (dolichos monocrop or maize-dolichos intercrop): one at the crop's centre (5 m from the outermost row) and the other at the border of vegetation (0.5 m from the outermost row). Aphid parasitoids were allowed to deposit their eggs on the plants for a period of seven days in the field plots. The sentinel plants were placed in mesh cages after being recovered and covered with a fine net mesh throughout transportation (Plate 4.3). The total number of live parasitoids, dead parasitoids, and aphid mummies was counted every three days, for thirty days. Aphids reproduce quickly and can be difficult to find in big cages, thus it was not practical to count the non-parasitic aphids, and instead we concentrated on standardising the initial aphid population and tracking the number of mummies that emerged.

Plate 4. 3: Aphid infested dolichos sentinel plant (A), covered sentinel plant (B) and sentinel plants transportation to greenhouse (C).

4.3.3 Bean aphids' severity, incidence and abundance

Data on aphid damage severity, abundance and percent incidence were collected in the same field trial described in sub-sections 3.3.6, 3.3.7 and 3.3.8.

4.3.4 Dolichos bean grain yield

Dolichos grain yield and yield components were recorded at maturity when pods turned brown. The pods were harvested separately from the middle rows falling within a sampling area of 36 m^2 for each treatment. Harvested lablab pods were sun-dried and threshed with the moisture content recorded using a digital moisture meter (Draminski S.A., Poland). At 13 % moisture content, grains from each treatment were weighed separately using a portable digital scale (Manufacturer: Comglobal Solutions, India) and converted to kg ha^{-1} using the following as described in equation 3.5.

4.3.5 Effect of field margin species on the abundance of *Aphidius colemani* parasitoid and control of bean aphid

Data on the abundance of *A. colemani* parasitoids and *A. fabae* bean aphids, pest/natural enemy mortality and parasitism were collected by counting the number of live, dead and mummified aphids inside the cage after every 3 days for 35 days with aid of a hand lens. The sentinel plants were carefully removed from the cages at the end of the 30 days and place on benches lined with a white cloth, and the final counts for aphid mummies and live and dead parasitoids using a hand lens.

4.4 Data analyses

To evaluate field margin vegetation and cropping systems with respect to conservation and improvement of bean aphid natural enemies, species diversity and evenness indices were applied. The need for knowledge on field margin vegetation and cropping systems in the conservation of natural enemies led to determination of distribution evenness of the insect family's composition. The number of natural enemies captured during the lablab's vegetative, flowering, and podding growth stages per field margin vegetation and cropping system served as the measuring unit. The Shannon-Weaver diversity index (H) and Pielou evenness index € were used to calculate the variation of the natural enemies (Pielou, 1966).

The number of aphid natural enemies counted across the different treatment par'meters were subjected to analysis of variance (ANOVA) with main independent variables: Cropping system, field margin vegetation, capture method and their interactions and the treatment means separated using Tukey's HSD. In the cage trials, ANOVA was carried out with field margin vegetation, cropping system, crop stage and their interactions as the independent variables. The abundance of fluorescent dye marked insects captured from each field margin weed species were compared against capture methods and their interactions by ANOVA with post-host LSD. Analyses were done using XLSTAT version 2019.2.2.59614 (Addinsoft, 2019). XLSTAT statistical and data analysis solution (Boston, MA, USA. https://www.xlstat.com).

4.5 Results

4.5.1 Abundance, diversity and temporal distribution of bean aphid natural enemies

A total of 2029 natural enemy individuals were collected.The invertebrate taxa that were collected were divided into two major groups of natural enemies: parasitoids and predators. Ten families of natural enemies, consisting of flying and crawling predators and parasitoids from six insect orders, were classified based on these categories: (Hymenoptera (Braconidae, Ichneumonidae, and Formicidae) (ants were listed as possible natural enemies because they can serve as pest controllers in tropical cropping systems). (Milligan *et al.*, 2016; Wielgoss *et al.*, 2014), Hemiptera (Nabidae, and Geocoridae), Diptera (Tachinidae, and Syrphidae), Coleoptera (Cocccinellidae, and Carabidae) and Neuroptera: (Chrysopidae) (Table 4.1). On the basis of relative percent abundance, Formicidae and Tachinidae were the most abundant families with 29.6% and 22.5%, respectively. The lowest abundance was observed in the Chrysopidae and Carabidae families with 3.1% and 3.5%, respectively (Table 4.1).

Table 4. 1: Relative abundance of natural enemies and other insect captured during the cropping season.

Category	**Order**	**Family**	**Counts**	**Relative abundance (%)**
Parasitoid	Hymenoptera	Braconidae	119	5.9
Parasitoid		Ichneumonidae	224	11.0
Other		Formicidae	601	29.6
Predator	Hemiptera	Nabidae	141	6.9
Predator		Geocoridae	99	4.9
Parasitoid	Diptera	Tachinidae	457	22.5
Predator		Syriphidae	146	7.2
Predator	Coleoptera	Coccinellidae	109	5.4
Predator		Carabidae	71	3.5
Predator	Neuroptera	Chrysopidae	62	3.1
Total			**2029**	**100**

Generally, the presence of field margin vegetation did not contribute to increased natural enemy taxonomic richness (ANOVA: $F_{(1, 18)} = 1.01$; $P=0.32$), abundance (ANOVA: $F_{(1, 18)} = 1.12$; $P=0.30$) or diversity (ANOVA: $F_{(1, 18)} = 0.01$; $P=0.91$). Cropping system revealed a significant difference only for taxonomic richness index for monocrop (8.33) versus intercrop (7.08) (ANOVA: $F_{(1, 18)} = 4.63$; $P=0.04$) with no significant differences between monocrops and intercrops in terms of natural enemy abundance (ANOVA: $F_{(1, 18)} = 3.69$, $P=0.07$) and diversity (ANOVA: $F_{(1,18)} = 2.85$; $P=0.11$). Natural enemy taxonomic richness (ANOVA: $F_{(2, 18)} = 3.48$; $P=0.05$) and abundance (ANOVA: $F_{(2, 18)} = 6.06$; $P<0.001$) altered over the course of the cropping season but there was no significant change in variation (ANOVA: $F_{(2, 18)} = 2.33$; *P=0.13*). The cropping system, dolichos growth stage, and vegetation along the field border all had an impact on the number of insect families that were captured, which varied from four to ten. The Shannon–Weaver index ranged between 1.81 and 1.01. The highest diversity index of 1.81 was noted in dolichos-maize intercrop at vegetative stage in the presence of field margin vegetation. In the absence of field margin vegetation, the lowest diversity index of 1.01 was observed in dolichos monocrop at podding stage (Table 4.2).

Table 4. 2: Mean abundance and diversity of bean aphid natural enemies as influenced by cropping system, field margin vegetation and dolichos growth stages.

Margin	**FMV present**						**FMV absent**					
Cropping	**Monocrop**			**Intercrop**			**Monocrop**			**Intercrop**		
Stage	**Veg**	**Flow**	**Pod**	**Veg**	**Flow**	**Pod**	**Veg**	**Flow**	**Pod**	**Veg**	**Flow**	**Pod**
Total insect families (*S)	10	7	9	9	6	8	8	9	8	7	5	8
Abundance	163	54	108	81	58	94	91	57	124	47	24	117
Shannon–Weaver index (H)	1.29	1.03	1.05	1.81	1.40	1.32	1.52	1.04	1.01	1.61	1.28	1.24
Evenness index (E)	0.57	0.55	0.46	0.85	0.80	0.63	0.73	0.48	0.51	0.83	0.82	0.59

Note; Veg=vegetative stage, Flow=flowering stage and Pod=podding stage.

4.5.2 Bean aphid natural enemy abundance as influenced by cropping system, margin vegetation and dolichos growth stage

Field margin vegetation significantly influenced the abundance of natural enemies (ANOVA: $F_{(1, 712)} = 0.70$; *P=0.04*). Similarly, the cropping system and the interaction between field margin vegetation and cropping system were significant on natural enemies abundance (ANOVA: $F_{(1, 712)} = 2.04$; *P=0.03* and $F_{(1, 712)} = 0.01$; *P=0.00,* respectively) (Table 4.3).

Table 4. 3: Mean population of natural enemies collected within dolichos crop grown as either a monocrop or intercrop (with maize).

Field margin vegetation	**Cropping system**	**Natural enemies**
Absent	Intercrop	2.08^{c}
	Monocrop	3.29^{b}
Present	Intercrop	2.76^{bc}
	Monocrop	4.10^{a}
Source of variation	**df**	**Natural enemies**
Margin vegetation	1	0.70
		0.04
Cropping system	1	2.04
		0.03
Margin vegetation×Cropping system	1	0.01
		0.00

Values in the same column followed by the same letter are not significantly different using Tukey's HSD test at P<0.05.

In relation to the cropping system, dolichos monocrop had the highest number of natural enemies (3.29-4.10) as compared to intercrop (2.08-2.76) (Table 4.3). The presence or absence of margin significantly influenced the abundance of some aphid natural enemies taxa. Higher abundance of natural enemies was observed in plots surrounded with the field margin vegetation compared to plots which had no field margin vegetation for both dolichos monocrop and dolichos-maize intercrop (Figure 4.2).

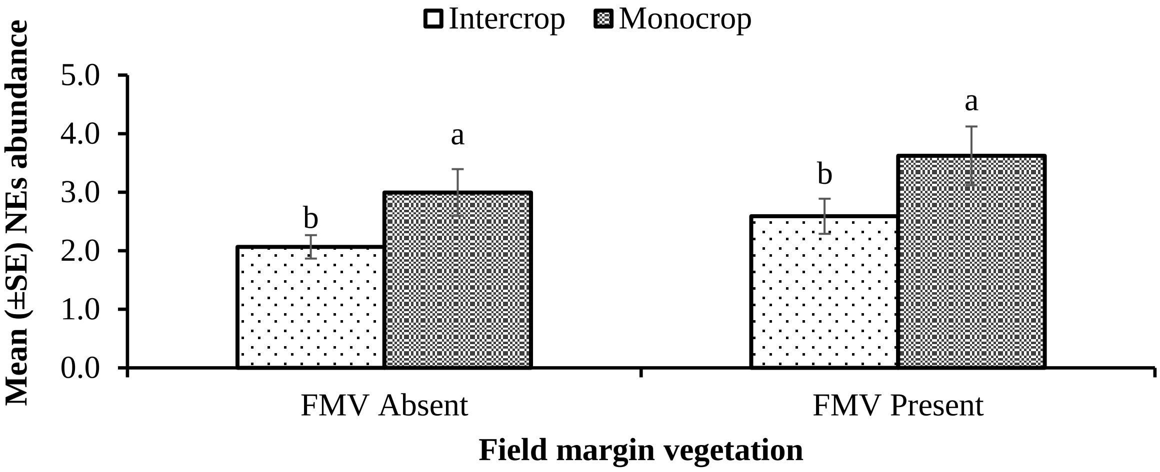

Figure 4. 2: Abundance (Mean±SE) of bean aphid natural enemies as influenced by cropping systems either with or without field margin vegetation.

Over the time of the study period, across the two seasons nine families of natural enemies were collected in the fields at all the three crop stages. Across the growth stages no natural enemy family had significant higher densities in more than one growth stage. The mean population abundance for most of the natural enemies was highest at the podding stage followed by the vegetative stage and the lowest population at the flowering stage (Table 4.4).

Table 4. 4: Mean abundance (±SE) of bean aphid natural enemies as influenced by dolichos growth stages.

Category	**Order**	**Family**	**Vegetative**	**Flowering**	**Podding**	**F-value**	**Pr > F**
Parasitoid	Hymenoptera	Braconidae	0.30±0.04a	0.07±0.02b	0.13±0.03b	5.31	0.01
Parasitoid		Ichneumonidae	0.35±0.06a	0.29±0.05a	0.30±0.06a	0.12	0.89
Predator	Hemiptera	Nabidae	0.16±0.03ab	0.13±0.03b	0.30±0.07a	5.95	0.003
Predator		Geocoridae	0.13±0.03ab	0.08±0.02b	0.20±0.04a	2.71	0.03
Parasitoid	Diptera	Tachinidae	0.84±0.12a	0.44±0.06b	0.63±0.10ab	3.98	0.02
Predator		Syriphidae	0.14±0.03a	0.15±0.05a	0.33±0.09a	3.12	0.07
Predator	Coleoptera	Coccinellidae	0.24±0.05a	0.13±0.03ab	0.08±0.02b	2.28	0.001
Predator		Carabidae	0.07±0.02a	0.14±0.04a	0.09±0.03a	1.05	0.35
Predator	Neuroptera	Chrysopidae	0.10±0.03a	0.02±0.01a	0.14±0.07a	2.75	0.06

Values in the same row followed by the same letter are not significantly different using the Tukey's HSD test at P<0.05.

4.5.3 Dye experiment results – interactions with margin plants

Insects with traces of fluorescent dye were observed in plots with and without margin plant species surrounding crop borders. Overally, out of 2029 of natural enemies that were collected, 201 had dye traces and 1828 had no dye traces representing 10% and 90%, respectively. *Galinsoga parviflora* exhibited the largest proportion (6.31%) among the 201 fluorescent dye designated natural enemies, whereas *Tagetes minuta* had the lowest proportion (0.20%) (Figure 4.3).

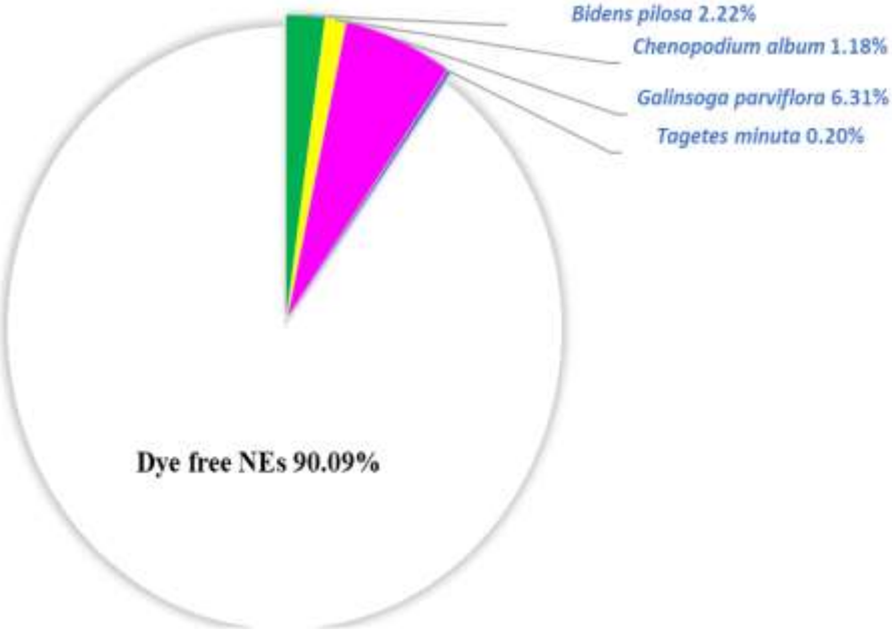

Figure 4. 3: Proportion of natural enemies with and without fluorescent dye captured. Florescent dye was applied to individual margin plant species that supported natural enemies where; yellow = *Chenopodium alba*, green = *Bidens pilosa*, pink = *Galinsoga parviflora* & blue = *Tagetes minuta*.

The natural enemy taxa demonstrated their preference in the field margin plants by carrying dye originating from the four distinct margin species (Figure 4.4), indicating potentially that different taxa have preferences for different plant species. All the families showed signs of visiting and interacting with the planted margin vegetation at least once, despite varying numbers and frequencies of visits. The Tachinidae family dominated the most visited plant species of *G. parviflora*, with 86 individuals. The species with at least three families that had the most visits were *B. pilosa* and *G. parviflora*. (Figure 4.4). Compared to *G. parviflora*, Ichneumonidae visited *B. pilosa* more frequently. The least visited plant species by Syrphidae visitors was *T. minuta* (Figure 4.4). Generally, bean aphid natural enemies and other insects were found to have visited all margin plants though at varying proportions.

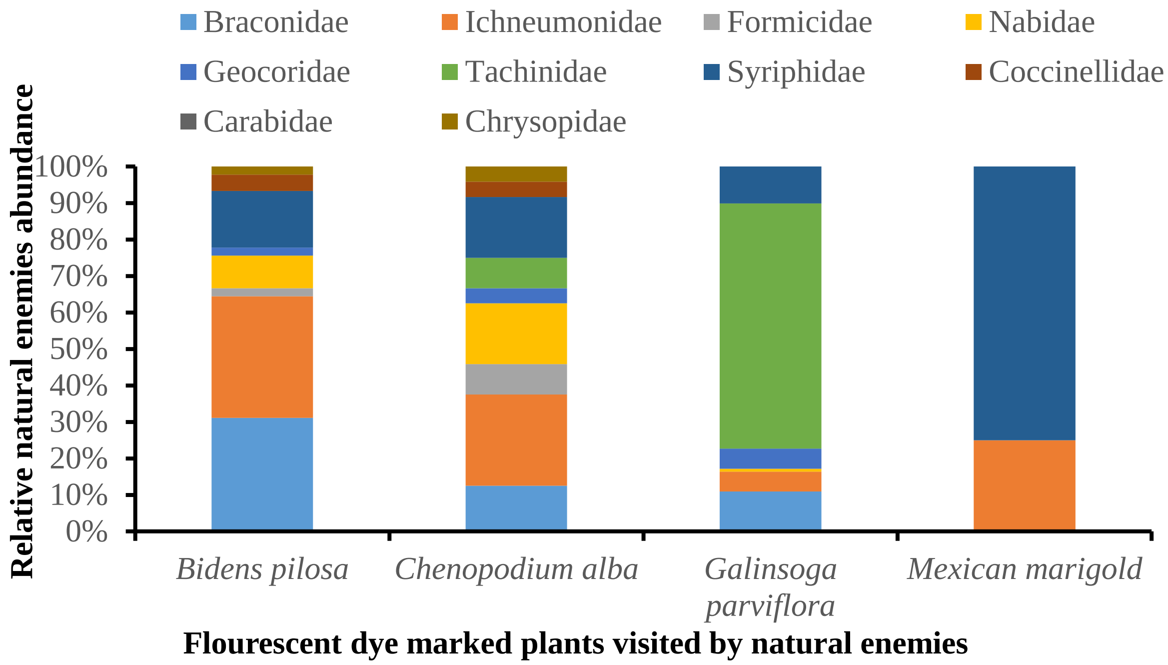

Figure 4. 4: Visitation of key groups of bean aphid natural enemies to field margin plant species.

4.5.4 Bean aphid parasitization

A total of 403 parasitoids emerged from the sentinel plants in the netted cages over a period of 31 days. Cropping system was not significant on the parasitization of the bean (ANOVA: F $_{(1,1476)}$ = 2.042; *P=0.153*). Monocrop had a higher number of parasitoids that emerged (54.1%) compared to the intercrop (45.9%). Field margin vegetation significantly influenced parasitization of the bean aphid (ANOVA: F $_{(1,1476)}$ = 43.905; *P<0.0001*). Sentinel plants that were placed in plots with field margin vegetation had a higher number of parasitoids (68.95%) compared to plots that had no field margin vegetation (31.1%). The three-way interaction of field margin vegetation, cropping system and crop growth stage indicated no significant difference for the emerged parasitoids (ANOVA: F $_{(1,1476)}$ = 0.058; *P=0.944*). The mean comparison revealed slight variations on the number of parasitoids that emerged across dolichos growth stages. At the podding and vegetative mean number of emerged parasitoids were 0.508 and 0.484, respectively. In relation to cropping systems and field margin vegetation, dolichos monocrop had a higher number of emerged parasitoids (Table 4.5).

Table 4. 5: Analysis of variance on the mean abundance of emerged parasitoids from dolichos sentinel plants planted as either a monocrop or intercrop (with maize) where field margin vegetation was either present or absent around the field plot.

Field margin vegetation	**Cropping system**	**Crop Stage**	**Parasitoids emerged**
Absent	Intercrop	Vegetative	0.290 bcd
		Flowering	0.089 fg
		Podding	0.081 g
	Monocrop	Vegetative	0.266 cde
		Flowering	0.161 defg
		Podding	0.121 efg
Present	Intercrop	Vegetative	0.484 a
		Flowering	0.315 bc
		Podding	0.234 cdef
	Monocrop	Vegetative	0.427 ab
		Flowering	0.274 cd
		Podding	0.508 a
Source of variation			**Parasitoids emerged**
R^2			0.052
F			7.351
Pr > F			**< 0.0001**
Field margin vegetation			43.905 **< 0.0001**
Cropping system			2.042 0.153
Crop stage			15.756 **< 0.0001**

Table 4.5: Continued…

Source of variation	Parasitoids emerged
Field margin vegetation × Cropping system	0.227
	0.634
Field margin vegetation × Crop stage	0.782
	0.458
Cropping system× Crop stage	0.748
	0.473
Field margin vegetation × Cropping system × Crop stage	0.058
	0.944

Values in the same column followed by the same letter are not different from each other at the 95% confidence interval using the Least Significant Difference test.

4.5.5 Dolichos bean grain yield

Results showed that dolichos bean grain yield was significantly affected by the presence or absence of field margin vegetation (ANOVA: $F_{(1, 12)} = 9.008$; *P=0.011*). Plots that were surrounded by field margin vegetation had a higher grain yield of 298.9 kg ha-1which was significantly different to plots which had no field margin vegetation (209.5 kg ha-1). Similarly, the cropping system was significant on grain yield (ANOVA: F (1, 12) = 5.179; P=0.042). Dolichos monocrop had a higher grain yield (288.1 kg ha-1) compared to maize-dolichos intercrop (220.3 kg ha-1). In relation cropping system and field margin interaction effect, dolichos monocrop in the presence of field margin vegetation had the highest grain yield (351.6 kg ha-1) (Figure 4.5).

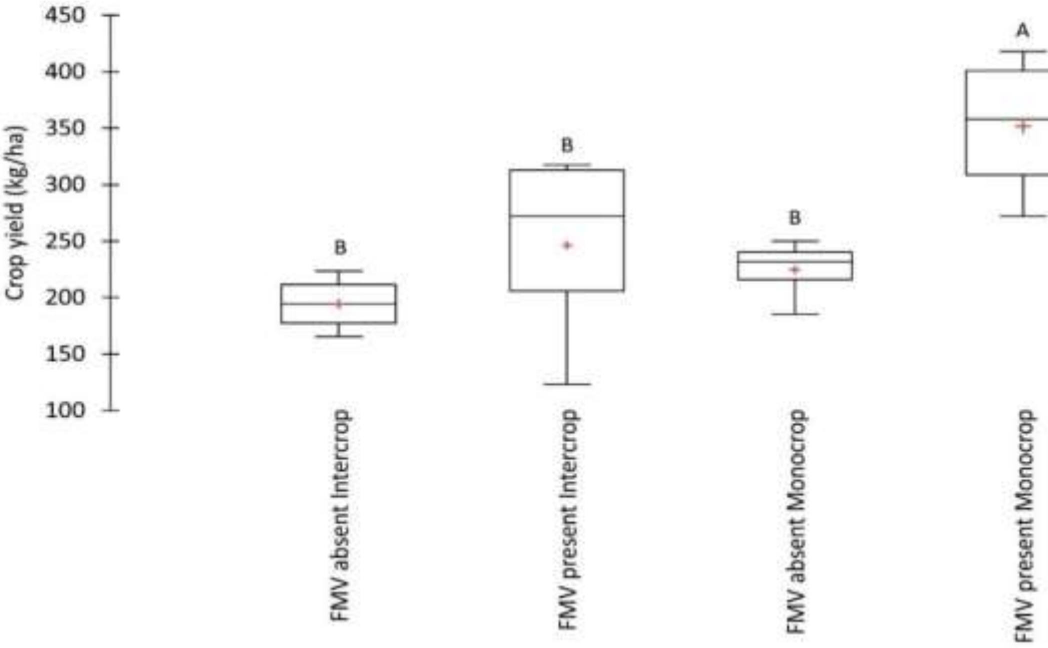

Figure 4. 5: Dolichos grain yield from monocrop and intercrop (with maize) when cropped with and without field margin vegetation (FMV).

4.5.6 The effect of field margin species on *Aphidius colemani* parasitoids and control of bean aphid

The presence margin plant resulted in a lower emergence of *Aphidius colemani* which was significantly different ($P<0.05$) from 10% sucrose (positive control) except for *Bidens pilosa*, which did not differ from in the number of live parasitoids from the controls. Results further revealed that the number of mummies found on bean plants in the cages was significantly influenced by the species of plant (ANOVA: $F_{(5,144)} = 13.088$; $P<0.0001$). The highest number of *Aphidius colemani* parasitoids (3.4) among plant species was observed in cages that had *Bidens pilosa*; this number was identical to that of water (3.7) and 10% sucrose (2.9). *Tagetes minuta* and *Galinsoga parviflora* were the plant species that least supported live parasitoids 1.6 and 1.1, respectively. In supporting aphid parasitization *Bidens pilosa* had the highest number of mummies (8.3) while the lowest number was obseved in *Tagetes minuta* (3.4) compared to 10% sucrose (11.0) and water (6.7) (Figure 4.6).

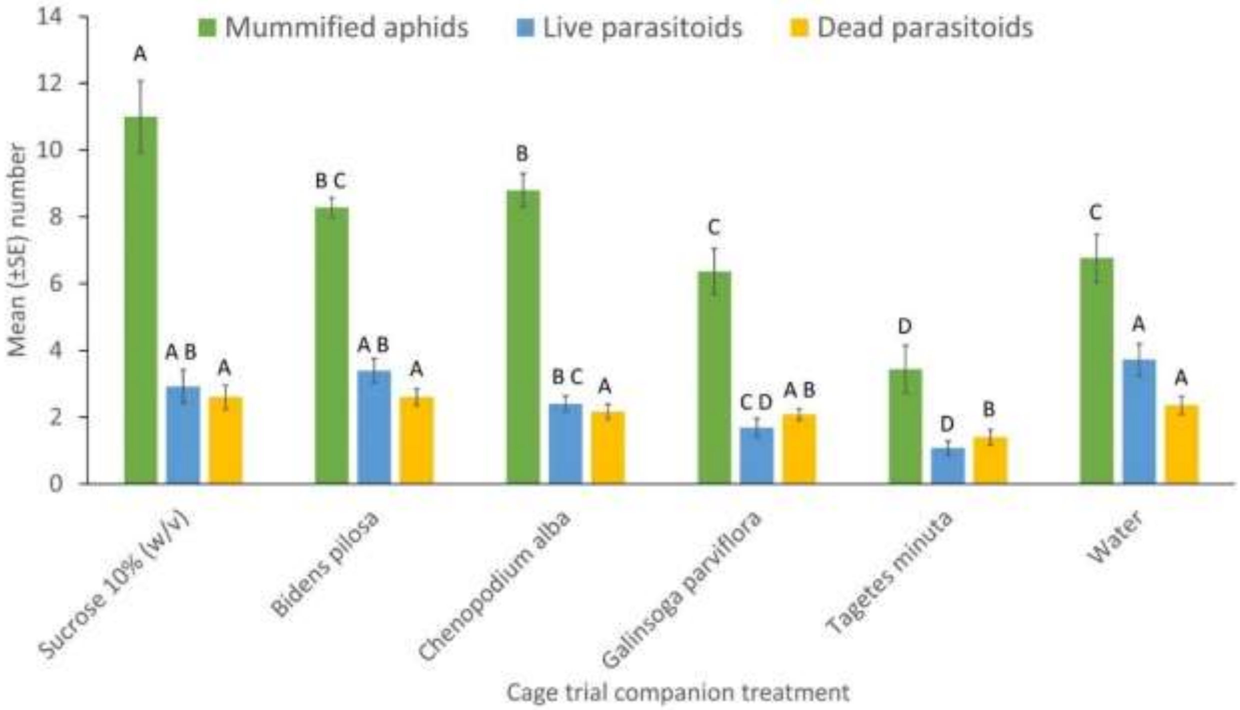

Figure 4. 6: *Aphidius colemani* emergence and parasitism as influenced by field margin plant species.

Bars represent the cumulative total number of mummies, live and dead parasitoids counted throughout the study period. Bars with the same fill pattern bearing the same letters are not significantly different usingTukey's HSD test at $P < 0.05$.

4.6 Discussion

This study demonstrated some beneficial effects of field margin vegetation on natural enemies with reduced aphid infestation in dolichos field plots and increased yield. The diversity and abundance of natural enemies were, however, limited to a few specific invertebrate taxa. The strongest positive effect was seen for Braconidae family, which are likely to be highly important in aphid control on dolichos. Intercropping seemed to provide no benefit for pest management of dolichos in relation to promoting natural enemy populations. Through tracking fluorescent dye applied to margin flowers, we found evidence of various natural enemies moving from margins into the dolichos crop, indicating that the presence of margin can provide resources for natural enemies which can move into the crop for natural pest regulation. The cage experiment revealed uncertain benefits of non-crop habitat in the provision of nectar resources in supporting parasitoid performance. This observations can be related to the foraging behavior of the parasitoids which varied with respect to supplemental food sources given (Abd El-Kareim *et al.*, 2007; Harvey & Fortuna, 2012; Kehrli & Bacher, 2008). In other studies, Wäckers and Fadamiro (2005) and Winkler *et al*. (2006) also reported that several parasitoids required a floral nectar source during their adult stages. Dolichos is a neglected crop in terms of pest management studies, but is growing in importance as a "future crop" (Minde *et al*., 2021). Therefore, understanding the natural enemy interactions within and around will be useful in developing sustainable pest management strategies in dolichos and other underutilised legumes. The study revealed that grain yield was greatly impacted by the presence of field margin vegetation around cropping field. This difference in yield could be attributed to margin vegetation facilitation natural enemies abundance and low number of insect pests (Middleton *et al*., 2021; Mkenda *et al*., 2015).

This study showed that presence of plant-rich field margins positively influenced natural enemy groups of Braconidae and Nabidae abundance. This finding is in agreement with the "Enemy Hypothesis" which predicts a positive correlation between plant species richness and natural enemy abundance (Bianchi *et al*., 2006; Root, 1973; Zhao *et al*., 2013). In this study, there was limited time for populations to build up and the founder populations would have been influenced by the composition of the wider landscape over a single season; on permanent smallholder fields the importance of non-crop habitat may be greater and affect more taxa this accords with studies such as (Mkenda *et al*., 2019; Mwani *et al*., 2021).

Understanding the pattern of natural enemies' movement into crops is critical in developing conservation biocontrol strategies (Costamagna *et al*, 2015; Schellhorn *et al*., 2014). This study observed visitation by natural enemies to the flowers of specific margin species before moving into the crop. The presence of florescent dye traces confirmed that natural enemies that could help protect the crop had previously visited margin plant species. This indicates that the natural enemies use the margin vegetation, and specifically the flowers, for enhanced support. *Bidens pilosa* and *Galinsoga parviflora* were the most visited plant species by the natural enemies. These plant species possess open flowers with highly accessible pollen and nectar, brightly coloured petals which are known to attract a wide range of insect families (Amaral *et al*., 2013; Kishinevsky *et al*., 2017). The finding is in line with results of the cage experiment where *B. pilosa* and *G. parviflora* supported emergence of high number of live parasitoids. Presence of nectar and pollen in these plants have been documented to significantly increase the activity, longevity and fecundity of predators and parasitoids (Bianchi & Wäckers, 2008; Ndakidemi *et al*., 2022; Parajulee *et al*., 1994). Conversely, *Tagetes minuta* showed little evidence of use by natural enemies captured from the crop, in contrast to other studies recording beneficial insect interactions with this plant (Souza *et al*., 2019). Most insect families collected in this study were in similar to those captured in related studies and included: Hymenoptera, Diptera, Coleoptera, Heteroptera and Neuroptera as these taxa are considered to have greater species richness of natural enemies (Vattala *et al*., 2006).

Other studies from tropical and temperate regions have reported increased abundance in wide a range of natural enemies groups such as parasitoids and spiders in non-crop land, adjacent vegetation or shelterbelts or farm holdings in agricultural farmlands (Perović *et al*., 2010; Schmidt *et al*., 2003). However, in this study field margin had significant impact on a limited number of families such as Braconidae and Nabidae. This observation can be attributed to the fact that most of the insect families are highly mobile and experimental plots were relatively close to each other, meaning this observation might be most pronounced in the less mobile groups where immediate surroundings are most important. Plants with floral resources such as *Ageratum conyzoides* which grow as weedy plants around farmlands have been documented to host several beneficial insects which can freely move into crops to suppress pest populations (Amaral *et al*., 2013; Amoabeng *et al*., 2020; Mkenda *et al*., 2019). In many cases, this is due to their provision of nectar and pollen, including outside the crop flowering period.

In this study, *Bidens pilosa,* a plant in the family of Asteraceae possesses easily accessible flowers with bright yellow centres, was the most preferred non-crop plant. The largest number of natural enemy individuals were observed to bear dye originating from *B. pilosa* compared to other margin plants; in addition, dye traces were found across a large number of insect families. *Galinsoga parviflora* was by also highly visited by natural enemies in the field; these plants show similar growth characteristics and stature (common, widely/naturally occurring, low growing with generalized flowers) around farmlands in Kenya. These results are in agreement with Amaral *et al.* (2013) who reported that the management of *Ageratum conyzoides* and *Bidens pilosa* weed species may provide an optimal strategy for the conservation of beneficial insects that utilize non-prey foods.

In relation to intercropping versus monocropping systems, reduced abundance of insect herbivores have been reported in intercrop systems, with higher yield from the mixture compared to monocrops (Malézieux *et al*., 2009). In the present study, the cropping system showed influence on natural enemy diversity and abundance, but contrary to expectations the dolichos monocrop was observed to have more natural enemies. This could have been enhanced by the ease with which natural enemies use olfactory, visual and acoustic cues to locate the bean aphids in dolichos monocrop, or the crop homogeneity assisting with movement through the plots. The low natural enemy occurrence in the intercrop could be associated with the “Resource Concentration Hypothesis,” (Root, 1973). The hypothesizes states that “the probability of herbivores finding their host plant, remaining on that plant and reproducing is higher in monocultures than in mixtures of several species, where the resource is diluted among other resources”.

Field margins established close to crops can enable more rapid arrival of natural enemies as nearby reservoirs for fast movement into the crop. The study findings showed that fluctuations in the populations of natural enemies is a function of time, this was influenced and reflected in the crop stage and season progression. Braconidae species were captured most in plots with no margin; one possibility is that, as small and light insects, they travelled from afar and arrived late compared with some of the larger and heavier insects. Colonization and population build ups were high at the beginning of the season (vegetation stage) as the natural enemies came into the ecosystem. The populations reduced as resources started declining at flowering and were very high at podding stage as resources increased exponentially. Although phenology and food quality of host may significantly influence the population development of the natural enemies, the general observation was that the population control and maintenance is a combined effect of biotic and abiotic factors. At the flowering stage of the crop which coincided with the flowering stage of the field margins; the natural enemy visits reduced into the crop most stayed at the margins. This could be associated with high availability of food resources such as nectar, pollen in the margin plants and reduced pest prey inside the crop because of increased competition for the available resources (Lu *et al*., 2014). Synchronization of the life cycle and environmental requirements of the pests and natural enemies is therefore important in determining an effective and sustainable biological control.

While the on-station trials showed that natural enemies were observed to be associated with the weedy margin species, the cage trials did not show much evidence on benefit of companion plants in supporting parasitoids. None of the plants outperformed where only water and aphid on sentinel plants was provided. *Tagetes minuta* was the worst performing treatment overall in terms of supporting parasitoid performance. The finding that *T. minuta* was a poor companion plant as well as being less visited by natural enemy groups implies the plant should not be prioritized as a margin plant within and around crop fields (Amoabeng *et al*., 2020; Kawuki *et al*., 2005).

The cage trial results indicated that the parasitoids in this experiment were not primarily limited by access to prey and non-prey resources (either because the insects were able to feed on aphid honeydew as a sugar energy source, or perhaps because the crop itself provides sufficient nectar; likely via extrafloral nectaries) (Marazzi *et al*., 2019). Benefits in the field may be more nuanced, such as shelter from weather conditions or refugia during non-cropping periods. One consideration is that some crops can, themselves, be beneficial resources for natural enemies during their flowering period. With a limited flowering period other resources (i.e., long-flowering wild plants) may be required outside this time in order to ensure sustainable natural enemy populations year-round. Weeds and trees around farmlands may play a particular role, especially in regions where the growing season is interrupted by either cold or dry periods.

The study demonstrated that high plant diversity has the potential to increase natural enemy abundance particularly of parasitoids. Natural enemies in the field use field margin plants before moving into the crop. Conversely, maize-intercrop may not assist in supporting natural enemy abundance and in fact, can be detrimental. Inclusion of field margin vegetation has potential in conservation of natural enemy diversity and abundance to promote integrated natural pest management and if adopted by smallholder farmers can reduce overreliance on synthetic insecticides. However, the extent to which individual field margin plant species provide support to specific natural enemy species requires more in-depth studies in order to generate precise and location-specific recommendations.

4.7 Conclusion

Results from this study revealed that *Bidens pilosa* supported the highest number of *Aphidius colemani* parasitoids. In addition, *Bidens pilosa* was most visited by the Ichneumonidae compared to *Galinsoga parviflora*. *Tagetes minuta* was the least frequented among the plant species. In both presence and absence of field margin vegetation, bean aphid natural enemies trapped had traces of fluorescent dyes. In relation to dolichos grain yield, higher grain yield was observed in dolichos monocrop compared to maize-dolichos intercrop. However, the cropping system was not significant on the parasitization of the bean aphids. In conclusion, these results show that *Bidens pilosa* can be very beneficial if planted on farm edges as field margin vegetation to enhance the population of predators and parasitoids. Plant species that have bright flowered petals can attract the natural enemies and serve as resting and reproductive places for the natural enemies.

CHAPTER FIVE

INFLUENCE OF FLORAL RESOURCE ABUNDANCE AND DIVERSITY IN SUPPORTING BEAN APHID NATURAL ENEMIES OUTSIDE MAIN GROWING SEASONS

Abstract

Populations of natural enemies of populations of insect pests are constantly declining, particularly in agricultural systems that involve the complete removal of plants after cropping seasons. Maintaining a diversity of non-crop flowering vegetation outside the cropping season may promote more reliable and efficient natural pest control. A study was conducted to determine the contribution of floral vegetation diversity and abundance on the conservation of natural enemies of bean aphids outside the dolichos main growing season. Field surveys on plant species and sampling of natural enemies were carried out across two locations (Njoro and Rongai) after harvesting dolichos monocrop and maize – dolichos intercrop on farmers' fields during the 2020 and 2021 (January - April) off-seasons. A total of 54 plant species were identified from the survey across Njoro and Rongai with 59% of them being annuals and 41% perennials. There was a significant seasonal variation in plant species richness (ANOVA: $F_{1,16} = 33.45$; $P<0.0001$) and diversity (ANOVA: $F_{1,16} = 7.20$; $P=0.0511$). While time after harvesting was a significant factor influencing the overall abundance of natural enemies (ANOVA: $F_{2,1133} = 8.11$; $P<0.0001$) they were generally higher in abundance in locations with margin plants or where a diversity of margin plants was observed. Natural enemies were most abundant in Njoro (52 %) compared to Rongai (48%), Formicidae and Carabidae as the most and least abundant families at 19% and 9%, respectively. These findings demonstrate that field floral plants can offer refuge for natural enemies and other beneficial insects outside the main cropping season. The conservation of natural enemies between crops may lead to efficient natural pest regulation early in the cropping season hence reducing reliance on insecticides application.

5.1 Introduction

Biological control of agricultural pests is focused on the conservation of natural enemies that move into crop fields for natural pest regulation that requires deliberate preservation and effective management of non-crop habitats around farmlands (Hatt *et al.*, 2017; Sorribas *et al.*, 2016). Natural or semi-natural flowering plants adjacent to croplands can be preserved or planted but the outcomes in supporting natural enemies are often crop pest and cropping system dependent (Landis *et al.*, 2000; Perović *et al.*, 2010; Ochieng *et al*., 2022). Non-crop vegetation can provide natural enemies with nectar, offer protection, and other prey, increasing their number and effectiveness in controlling pests (Bianchi & Wäckers 2008). In a study by Lundgren (2009), fecundity of female predatory and parasitic arthropods was attributed to the amount of food available at the adult stage. In the absence of flowering plants, the female parasitic wasps may reabsorb eggs and devote more energy to host seeking and survival, decreasing their ability to reproduce (Kishinevsky *et al*., 2017). Therefore, cropping systems that include complete removal of biomass at harvest create conditions that hinder resilient and effective natural enemy populations thus reducing their potential to control pests (Nilsson *et al*., 2016). Crop harvesting causes rapid changes in the structure of habitats, making agroecosystems unstable for natural enemies.

There are several groups of natural enemies known to provide control of aphid insect pests (Schmidt *et al*., 2003). These include parasitic wasps and larvae of syrphid flies, which feed on the aphids exerting natural control of aphids in the fields. The parasitic wasps are highly specific because they are able to locate aphid colonies from a greater distance via “alarm signals” emitted by an infested plant. After locating the aphid, the wasps use their ovipositor to lay eggs (oviposit) into the aphid abdomen where they grow inside and kill the aphid. More generalist natural enemies that also prey on aphids include carabid and staphylinid beetles and spiders, which mainly colonize plants from the ground (Schmidt *et al*., 2003). The other important natural enemies for aphids include lady beetles, lacewings; big eyed damsel and minute pirate bugs. These are predators and directly consume or feed on one or more aphid species (Desneux & Ramirez-Romero, 2009; Dixon, 2000). In addition, birds represent top predators for insects in many agricultural systems (Milligan *et al*., 2016). The adult of lacewings and hoverflies feed on nectar and pollen so floral resources are important in addition to soft bodied insects such as aphids (Lahiru & Costamagna, 2019). In natural field settings, ladybird beetles are frequently used as biological control agents against aphids. Aphid outbreaks and densities will be delayed and avoided (Heimpel & Asplen, 2011). They regulate aphid populations as they are ravenous with good searching ability, a large capacity for consuming both adults and larvae stages and high rates of reproduction (Amorós-Jiménez *et al*., 2012). The carabids are generally polyphagous; however, they are also voracious feeders, consuming nearly their own body mass in food each day. Their feeding habits are specific, and they primarily consume bean aphids that land on the ground.

Although certain invertebrate species have evolved a wide range of adaptive mechanisms in response to changes in habitat structure(Gavish-Regev *et al*., 2008; Langellotto & Denno, 2004), Research by Opatovsky and Lubin (2012) revealed that creatures that migrate may seek refuge in adjacent non-crop habitats, but small arthropods that live in crops, such spiders, may not be able to swiftly adapt to unforeseen changes in habitat quality as a result of harvestAdditionally, the study demonstrated that the majority of insect pests have short life cycles that coincide with the cropping season in order to adapt to these changes in habitat structure. However, natural enemies such as predators and parasitoids typically have a longer life cycles than seasonal crop growth cycles, prompting the necessity to access the availability of non-crop habitats that can sustain these arthropod groups (Menalled *et al*., 2003).Natural enemy abundance distribution patterns have been demonstrated to vary over the course of the crop's growth cycles (Kishinevsky *et al*. (2017) reported that the abundance of parasitoids was higher in natural habitats early in the growing season than it was in crops later in the growing season.

Similar seasonal patterns were previously demonstrated for spiders in desert wheat fields and were explained by the migration of spiders into the crop field throughout the season, combined with the high reproductive rates within the crop fields (Gavish-Regev *et al*., 2008). Studies by Gavish-Regev *et al*. (2008) and Opatovsky and Lubin (2012) indicated that harvest of annual crops leaves the fields largely bare of vegetation, and this can negatively affect natural enemies and other arthropods. Changes within crop habitats are considered to be less suitable for arthropods and may lead to either high mortality or to dispersal of crop dwelling arthropods into neighbouring habitats (Costamagna *et al*., 2015; Gontijo, 2019; Yang *et al*., 2017).

Many natural enemies multiply in response to the availability of the food hence there is a time lag between pests and natural enemy fluctuations. Natural enemies are highly mobile; their movement in and out of the crop will depend on how far their refuges are and the availability of prey and food. Therefore, throughout the season there will be fluctuation in population hence the need to assess their dynamics at several crop stages (Zhao *et al*., 2013). It is worth remembering that as plant communities change the associated organism species and population also changes, in this case as the crop stages and margin species change so do the natural enemies and pests and this influences their succession and colonisation (Chaplin-Kramer *et al*., 2011). The crop vegetation is short-lived and must be recolonized by natural enemies at the beginning of each crop season, meaning that maintaining viable natural enemy populations in non-crop habitat outside the growing system is important to achieving reliable biocontrol without resorting to synthetic pesticides. To successfully build suitable non-crop habitat, a detailed understanding of the distribution, diversity and abundance of the plant species and how this links to plant–arthropod diversity after crop harvesting is vital. It is important that the non-crop habitat supports natural enemies preferentially over crop pests, or the benefits for natural pest regulation may be limited. This study aimed to measure how the abundance and diversity of field margin plants supported the abundance of natural enemies of bean aphid (*Aphis fabae*) between cropping season in a legume crop, lablab (*Lablab purpureus*) under different agro ecological conditions.

5.2 Materials and methods

5.2.1 Study site

This study was conducted during February-April 2020 and January-March 2021 succeeding the main cropping seasons of May-December 2019 and March -November 2020, respectively in sixteen farmers' fields in Nakuru County: eight in Njoro and eight in Rongai, sub-counties. Njoro sub-county is located at 0° 10′ - 0° 29′ S and 34° 7′ - 34° 20′ E with an altitude range of 2000 to 2500 metres above sea level (m.a.s.l). The annual rainfall range is 1000 to 1250 mm and temperature ranges from 17° C to 30° C. Rongai is located at 0° 10′ - 0° 29′ S and 34° 7′ - 34° 20′ E with an altitude range of 1480 to 1550 m.a.s.l. The annual rainfall ranges of between 750 to 1000 mm and temperature range of 19° C to 32° C. The Njoro soils are well drained dark reddish clays, classified as Mollic Andosols whereas Rongai soils are well drained sandy clay loams, classified as Vitric Andosols (Jaetzold *et al.*, 2012).

5.2.2 Experimental design and treatment application

The experimental design is as described in chapter 3, section 3.2.2.

5.3 Data collection

5.3.1 Sampling of natural enemies of bean aphids (*A. fabae*)

The sampling of natural enemies was done in field margins near experimental plots that were either planted with lablab monocrop or lablab-maize intercrop. Sampling was done monthly for three months after harvesting the field crops. Trapping of natural enemies was conducted using yellow pan traps for ground dwelling insects, yellow sticky cards and sweep nets for flying insects. For pan trapping, yellow plastic pans measuring 20 cm in diameter and

5 cm high were placed at the ground level to collect natural enemies. The pans were filled with a premixed liquid solution containing 250 ml of water, 5 g of salt to preserve the natural enemies and 5 ml of odourless liquid detergent to break the surface tension of the water. The traps were left in the field for 48 hours. Thereafter, the trapped insects were retrieved by washing with clean water and sieving. The insects were picked from the sieve using a camel hairbrush and placed in 50 ml plastic falcon tubes filled with 25 ml of 75% ethanol for preservation before being taken to the laboratory. The insects were placed under a dissecting microscope (Leica ZOOM 2000 Inc. Buffalo, NY U.S.A 14240-0123) at 200× magnification for counting and identification up to the family level using Simon and Schuster's identification key (Arnett & Jacques, 1981).

Sticky trap sampling was done by hanging yellow sticky cards (8 cm width x 24 cm length) 1 m above the ground level at the field margin for the two cropping systems. The sticky traps were later collected from the field after 48 hours, placed in non-sticky lamination pouches of 25 cm x 10 cm and taken to the laboratory for identification up to family level. Sweep net sampling was used to capture the natural enemies in the field margins according to Spafford and Lortie (2013). Sampling involved moving forward along the field margin vegetation and making 10 sweeps parallel within the margin. The sweep net bag was closed immediately and the insects caught were carefully transferred to a jar containing cotton wool soaked with formalin where they were left for 2 hours. The preparation and identification of the insects were the same as for pan and sticky trapping. The sampling of the natural enemies was done four times during the off-season period under different field conditions post harvesting dolichos monocrop and maize-dolichos intercrop (Plate 5.1).

Trapping inside previous crop area

Trapping in ploughed area

Trapping inside another crop - Cassava

Trapping near crop a residue burnt

Trapping inside previous crop area turned into grazing paddocks

Trapping near a newly planted crop – common bean

Plate 5. 1: Placement of traps under different field conditons after harvesting dolichos monocrop and maize-dolichos intercrop.

5.3.2 Sampling of plant species composition and abundance

The on-season experiments were left bare after harvesting lablab and lablab maize intercrop and were not considered during sampling. The plant species composition in the field margins was determined by randomly throwing a 1 m^2 quadrat three times along the field margin. The plant species within the quadrat were identified and each species abundance was expressed as a percentage of the total number of plants. All the individual field margin plants that were in the quadrat were counted and the ratio of one species to the total count of the field margin plants as described in equation 3.2.

5.4 Data analyses

Data on plant species and natural enemies' counts were subjected to arcsine and square root ($\sqrt{x+1}$) transformation, respectively to correct for heterogeneity of treatment variances. For plant species the unit for calculations was the number of plants counted one month after harvesting lablab crops. For natural enemies the unit for calculations were the number of natural enemy groups caught using sticky traps, pan traps and sweep nets at different months post-harvesting lablab crop. To determine extent and how field margin vegetation conserve bean aphid natural enemies outside lablab main growing season, a species diversity index was calculated. To establish the diversity of either natural enemies or margin plants two of the most commonly used indices, Shannon-Wiener index and Simpson's species diversity, respectively, were adopted to quantify the diversity indices for each category. The diversity of the natural enemies was determined using the Shannon-Wiener index of diversity (H) calculated at family-level for assessing landscape influence on the abundance of insect predators in rice (Khan, 2015). Previous studies have demonstrated that calculation of diversity index at family-level is an appropriate proxy for species-level H (Osborne *et al.*, 1980; Zou *et al.*, 2020) and this is more practical when data sets are large or the ecosystem is understudied. Field margin plant species diversity was determined using the Simpson's species diversity index.

The effects of cropping season, location, month after harvesting and their interactions for natural enemy population, species richness and diversity were subjected to analysis of variance (ANOVA) using the procedure for general linear model in SAS Institute version 9.4 (SAS Institute, 2011). Similarly, season, location and their interaction for margin plants populations, species richness and diversity, aphid infestation and grain were subjected to ANOVA (Equation 5.1).

$$Y_{ijklmn} = \mu + S_i + L_j + SL_{ij} + T_k + ST_{ik} + LT_{jk} + C_l + SC_{il} + LCj_l + TC_{kl} + SLTC_{ijkl} + F_m + \mathcal{E}_{ijklm}$$

Equation 7.5

Where; Yijk= Response variable; µ = Overall mean; S_i = Effect due to the i^{th} season; L_j = Effect due to the j^{th} location; SL_{ij} = Interactive effect of the i^{th} season and the j^{th} location; T_k = Effect due to the k^{th} sampling time; ST_{ik} = Interactive effect of the i^{th} season and the k^{th} sampling time; LT_{ik} = Interactive effect of the j^{th} location and the k^{th} sampling time; C_l = Effect due to the l^{th} cropping system; SC_{il} = Interactive effect of the i^{th} season and the l^{th} cropping system; LC_{il} = Interactive effect of the j^{th} location and the l^{th} cropping system; TC_{kl} = Interactive effect of the k^{th} sampling time and l^{th} cropping system; $SLTC_{ijkl}$ = Interactive effect of the i^{th} season, j^{th} location, k^{th} sampling time and l^{th} cropping system; F_m = Effect due to the m^{th} farm/replicate and $\mathcal{E}_{ijklm}$=random error term.

The treatment means were separated using Tukey's Honestly Significant Difference (HSD) test at $P < 0.05$. Simple linear correlation analysis was carried out to determine relationships between the diversity of margin plants and populations of natural enemy months after crop harvest.

5.5 Results

5.5.1 Abundance and diversity of plant species at the field margins

A total 54 plant species were observed from the field survey across Njoro and Rongai during the study periods. There was a significant seasonal variation for species richness (P<0.0001) and species diversity (P<0.05) (Table 5.1). A higher species richness was observed during 2021 in Njoro (12.25) and Rongai (13.86) as compared to 2020 (Njoro: 5.75; Rongai: 6.75). The higher species richness in 2021 off season supported diversity of the natural enemies where a higher Simpson's diversity index was observed during the same period. In relation to species richness and diversity, Rongai had higher species richness and diversity compared to Njoro for both 2020 and 2021 off seasons (Table 5.1). The interaction effect due to location and season was not significant for species richness and diversity. However, higher species richness was observed in Rongai which was consistent both in 2020 and 2021 (Table 5.1).

Table 5. 1: Plant species richness and species diversity in Njoro and Rongai during 2020 and 2021 January to April off- cropping seasons.

Off-cropping season	**Location**	**Species richness**	**Simpsons' diversity index**
2020	Njoro	5.75b	0.67a
	Rongai	6.75b	0.70a
2021	Njoro	12.25a	0.79a
	Rongai	13.86a	0.78a
R^2		0.764	0.398
F		5.166	1.057
Pr > F	Df	0.002	0.444
Season	1	33.57***	4.43*
Location	1	1.786	0.011
Season × Location	1	0.189	0.126
Farmer/Replicate	7	0.839	0.934

Df: degree of freedoms; Pr > F: Probability greater than F_{calc}

Values in the same column followed by the same letter are not different from each other at the 95% confidence interval using the Tukey's Honestly Significant Difference (HSD) test.

In this study results revealed that 59% of the margin species were annuals and 41% perennials. In Njoro annual species were the most abundant during the 2020 off-season compared to perennials during the same period (Figure 5.1). In Rongai, perennial plant species were more abundant than annuals during the 2020 off cropping season. During the 2021 season, low abundance of the plant species was observed across the two locations. Rongai had the lowest abundance of perennial plant species (5%) compared to Njoro (6%). Annual plants across the two locations were at 8% during the 2021 off-season (Figure 5.1).

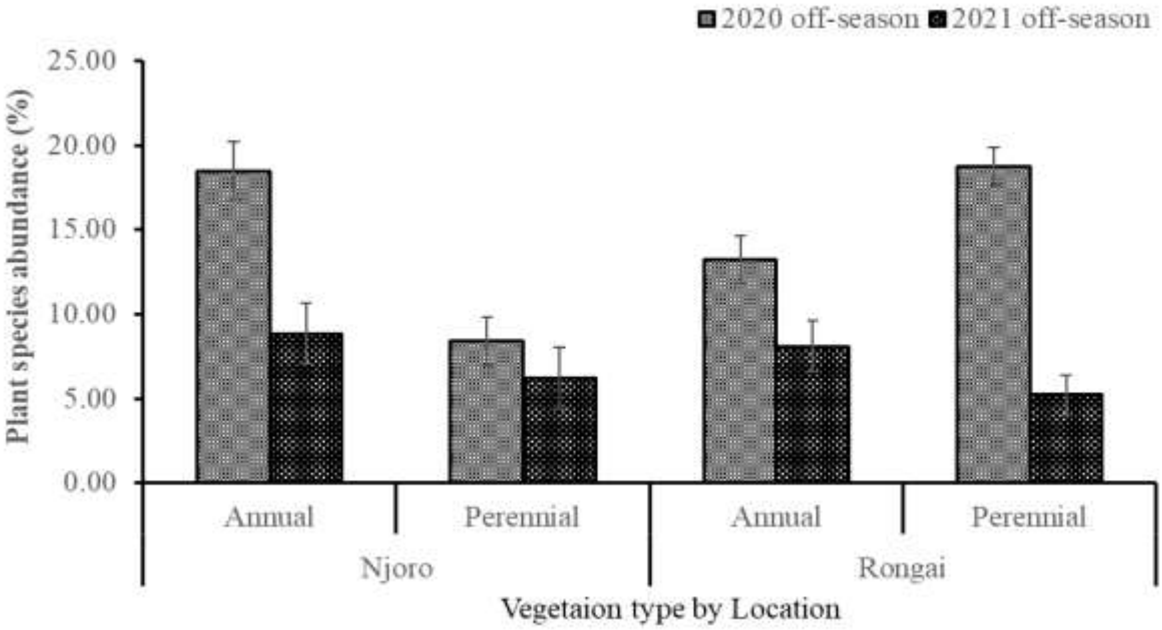

Figure 5. 1: Mean plant species abundance (percentage) in Njoro and Rongai during 2020 and 2021 off- cropping seasons.

Vegetation type here means life cycle of plant species within the margins.

In Njoro during the 2020 off season, the most abundant plant species were *Conyza bonariensis* (55%), *Bidens pilosa* (33%), *Ageratum conyzoides* (28%) and *Achyranthes aspera* (20.50%) with all the species categorized as annual plants. Among the perennial plants, the most abundant were *Lantana camara* (13%) and Tephrosia *vogelii* (10%). In Rongai, the most abundant annual plants were *Centaurea melitensis* (35%), *Galinsoga parviflora* (28%) and *Ageratum conyzoides* (22%). For the perennial plants, the most abundant were *Phaponticum repens* (44%), *Teraxacum officinale* (41%), *Richardia brasiliensis* (30%) and *Ocimum gratissimum* (20%) (Table 5.2).

Table 5. 2: Abundance (%) of field margin plants at Njoro and Rongai during the 2020 off-season (January-March 2020).

	Njoro		Rongai	
Plant species/Growth cycle	**Annual**	**Perennial**	**Annual**	**Perennial**
Achyranthes aspera	20.50	-	-	-
Ageratum conyzoides	27.50	-	21.71	-
Alternanthera pungens	-	-	-	3.00
Amaranthus hybridus	3.00	-	5.00	-
Anagallis arvensis	6.00	-	5.83	-
Bidens Pilosa	33.00	-	10.20	-
Centaurea melitensis	-	-	35.00	-
Chenopodium album	10.00	-	-	-
Commelina benghalensis	13.40	-	4.00	-
Conyza bonariensis	55.00	-	1.50	-
Crossocephelum rubens	-	7.00	-	-
Cyanthilium cinerum	6.67	-	18.33	-
Cyathula polycephala	-	-	6.00	-
Galinsoga parviflora	20.20	-	28.00	-
Lantana camara	-	13.00	-	8.50
Leonotis nepetifolia	6.33	-	20.33	-
Ocimum gratissimum	-	-	-	20.00
Oxygonum sinuatum	-	-	1.00	-
Parthenium hysterophorus	-	-	-	9.00
Phaponticum repens	-	-	-	44.00
Physalis anguleta	4.50	-	-	-
Raphanus raphanistrum	-	-	3.00	-
Richardia brasiliensis	-	-	-	30.00
Silene gallica	-	-	2.00	-
Solanum incanum	-	-	-	13.50
Sonchus arvensis	-	-	10.00	-

Table 5.2: Continued…

	Njoro		Rongai	
Plant species/Growth cycle	**Annual**	**Perennial**	**Annual**	**Perennial**
Tagetes minuta	10.60	-	12.43	-
Tephrosia vogelii	-	10.00	-	-
Teraxacum officinale	-	-	-	41.00
Verbena bonariensis	-	6.00	-	5.50

During the 2021 off-season, the most abundant annual plant species in Njoro were *Ageratum conyzoides* (29%) and *Bidens pilosa* (23%). For the perennial species, the most abundant were *Setaria verticillate* (13%) and *Verbena bonariensis* (12%). In Rongai, the most abundant annual species were *Ageratum conyzoides* (26%), *Bidens pilosa* (20%), *Commelina benghalensis* (13%) and *Cyanthilium cinerum* (13%). For the perennial species *Richardia brasiliensis* (25%) *and Dathonia spicata* (11%) were the most abundant (Table 5.3).

Table 5. 3: Abundance (%) of field margin plants in Njoro and Rongai during the 2021 off-cropping season (January-March 2021).

	Njoro		Rongai	
Plant species/Growth cycle	**Annual**	**Perennial**	**Annual**	**Perennial**
Acacia nilotica	-	-	-	1.00
Acanthospermum hispidum	-	-	-	5.30
Achyranthes aspera	11.67	-	-	-
Ageratum conyzoides	28.67	-	25.36	-
Amaranthus hybridus	4.67	-	8.00	-
Aristida stricta	-	3.00	-	2.00
Asystasia schimperi	-	-	2.00	-
Avena fatua	3.00	-	-	-
Bidens Pilosa	22.67	-	19.91	-
Chloris Gayana	-	4.75	-	1.80
Commelina benghalensis	3.50	-	13.33	-
Conyza bonariensis	11.08	-	12.00	-

Table 5.3: Continued…

Plant species/Growth cycle	Njoro		Rongai	
	Annual	**Perennial**	**Annual**	**Perennial**
Crotalaria retusa	1.57	-	1.25	-
Cucumis myriocapus	1.00	-	4.00	-
Cyanthilium cinerum	5.80	-	12.83	-
Cynodon dactylon	-	5.00	-	2.57
Cyperus rotundus	-	1.00	-	1.33
Dathonia spicata	-	2.00	-	10.75
Datura stramonium	2.17	-	0.83	-
Digitaria scalarum	-	6.10	-	2.45
Digitaria velutina	-	-	-	3.50
Eleusine indica	-	-	2.00	-
Galinsoga parviflora	7.00	-	3.83	-
Hyparrhenia rufa	-	1.00	-	9.00
Lactuca virosa	7.20	-	1.00	-
Lantana camara	-	-	-	1.33
Leonotis nepetifolia	3.17	-	6.50	-
Leucas martinicensis	4.33	-	1.95	-
Ocimum gratissimum	-	-	-	1.00
Oxalis latifolia	2.83	-	1.80	-
Oxygonum sinuatum	1.50	-	-	-
Pennistum clandestinum	-	2.00	-	2.00
Physalis anguleta	5.71	-	-	-
Physalis ixocarpa	-	-	1.13	-
Poa annua	-	3.00	-	3.00
Portulata oleracea	-	-	3.00	-
Raphanus raphanistrum	6.00	-	2.17	-
Richardia brasiliensis	-	-	-	25.50
Senecio inaequidens	-	3.00	2.13	-
Setaria verticillate	-	12.50	-	2.67
Shckubria pinnata	-	-	1.50	-

Table 5.3: Continued…

Plant species/Growth cycle	Njoro		Rongai	
	Annual	**Perennial**	**Annual**	**Perennial**
Silene gallica	-	-	6.50	-
Sochus oleraceae	-	-	1.00	-
Solanum incanum	-	-	-	4.17
Solanum villosum	2.00	-	-	-
Sonchus oleracea	-	-	1.25	-
Spagila arvensis	9.50	-	7.00	-
Tagetes minuta	1.33	-	5.00	-
Teraxacum officinale	-	-	7.50	-
Trichodesma zeylanicum	-	-	-	1.00
Verbena bonariensis	-	12.00	-	3.75

5.5.2 Distribution, abundance and richness of natural enemies during off-seasons

The abundance of natural enemies changed over time after harvest (ANOVA: $F_{2,1133}$ = 8.11; *P<0.0001*). Natural enemy abundance did not differ significantly between the two locations, or between 2020 and 2021(ANOVA: $F_{1,1133}$ = 1.26; *P=0.262*) (Table 5.4).

Table 5. 4: Analysis of variance for overall abundance of natural enemies captured in Njoro and Rongai during 2020 and 2021 off seasons.

Source of variation	**DF**	**F-value**	**Pr > F**
Season	1	1.26	0.262
Location	1	1.79	0.181
Months after harvest	2	8.11	0.000**
Rep	7	1.47	0.174
Season ×Location	1	0.00	0.960
Season × Months after harvest	2	0.10	0.906
Location × Months after harvest	2	0.25	0.778
Season × Location × Months after harvest	2	0.10	0.905

Df: degree of freedoms; Pr > F: Probability greater than F_{calc}

A total of 9,355 potential natural enemies of bean aphids belonging to nine families were collected across the two locations in 2020 and 2021 off seasons. During the 2020 off-cropping season, 4,859 individuals were collected, with Njoro having 2,511 and Rongai 2,348. In relation to the individual count of trapped insects, Njoro over the three months after harvesting, the highest natural enemy numbers were collected two months after harvesting (999) and lowest at the third month (673). In Rongai, a similar trend was observed where the highest NEs were collected in the second month (900) and lowest at the month (562). During the 2021 off-season, a total of 4,496 individuals were collected. Njoro had a total of 2,393 with the highest NEs collected in the second month (957) and lowest at the third month (621). In Rongai, a total of 2,103 individuals were collected with the highest NEs in the second month (822) and lowest in the third month (546) (Table 5.5 and Figure 5.2).

Table 5. 5: Natural enemy abundance: The total numbers of families captured in Njoro and Rongai during the 2020 and 2021 off-cropping seasons.

NEs Families	**Njoro**				**Rongai**			
	2020 season	**2021 season**	**Total**	**% Abundance**	**2020 season**	**2021 season**	**Total**	**% Abundance**
Braconidae	74	135	209	4.26	83	162	245	5.50
Ichneumonidae	216	225	441	8.99	241	189	430	9.66
Formicidae	519	248	767	15.64	699	325	1024	23.01
Nabidae	184	493	677	13.81	152	479	631	14.18
Geocoridae	481	135	616	12.56	231	189	420	9.44
Tachinidae	269	339	608	12.40	347	167	514	11.55
Syriphidae	275	588	863	17.60	231	386	617	13.86
Coccinellidae	72	156	228	4.65	106	130	236	5.30
Carabidae	421	74	495	10.09	258	76	334	7.50

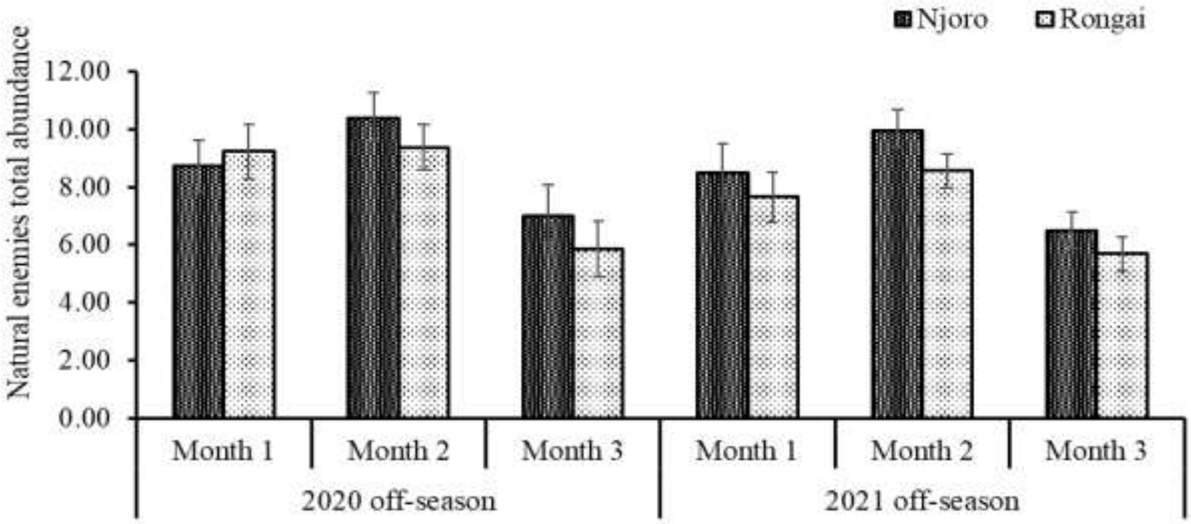

Figure 5. 2: Total abundance of natural enemies (Mean ± SE) in Njoro and Rongai during the 2020 and 2021 off-cropping seasons.

In the two locations, the most abundant families were Formicidae 1791 (19.14%) followed by Syriphidae 1480 (15.82 %) and the least abundant families were Braconidae 485 (4.85 %) and Carabidae 829 (8.86 %). During the 2020 off-season, the highest abundance of NEs one month after harvesting in Njoro were from the families of Geocoridae (2.85) and Carabidae (2.64), which changed to Formicidae (5.16) in the second month and Geocoridae (2.38) in the third month. During the 2021 off-cropping season, Syriphidae (2.87) and Formicidae (2.53) were the most abundant in the first month after harvesting. In the second month Nabidae (3.32) and Syriphidae (2.24) were the most abundant and changed to Syriphidae (1.45) and Tachinidae (1.43) at the third month (Figure 5.3).

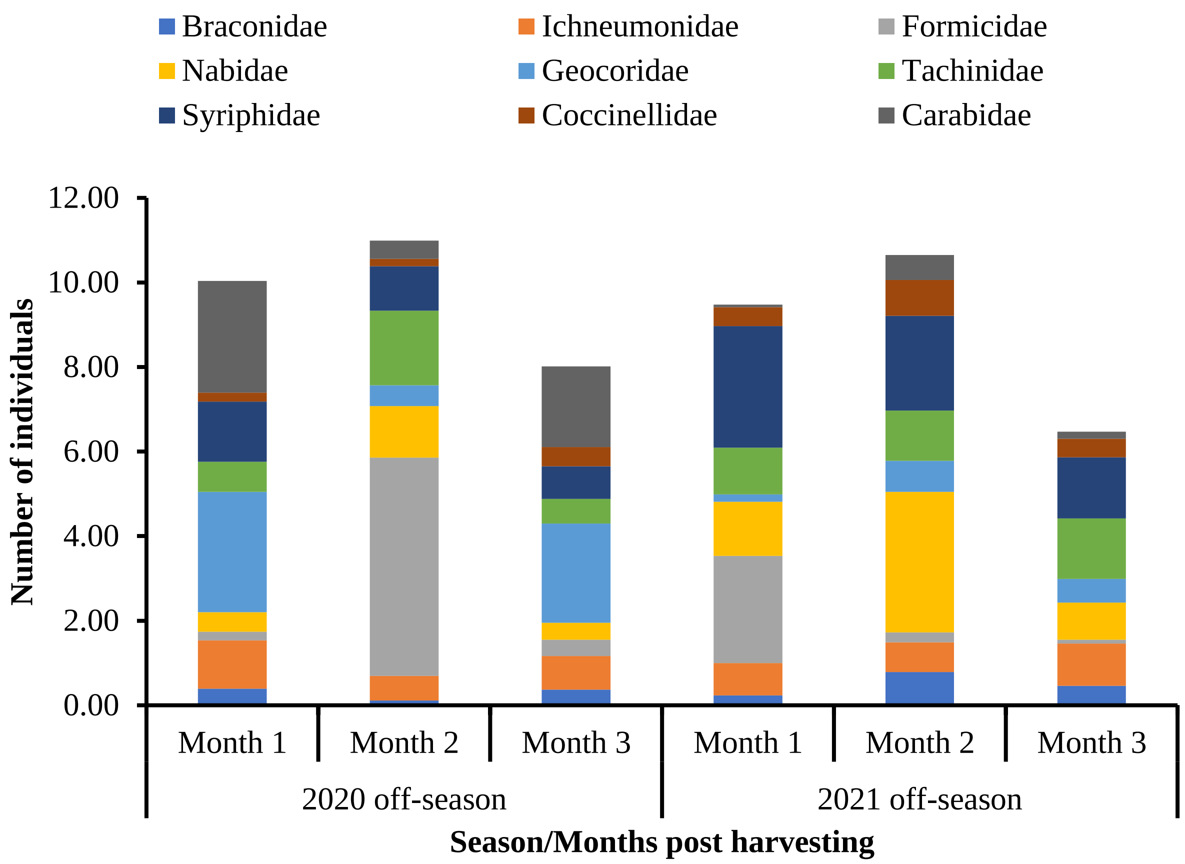

Figure 5. 3: Total abundance of natural enemy families (Mean ± SE) in Njoro months after dolichos harvesting during 2020 and 2021 off-seasons.

In Rongai during the 2020 off-season, the highest abundance one month after harvesting was observed in Formicidae (3.87) while Formicidae (2.39), Carabidae (1.60) and Geocoridae (1.38) were the most abundant second month and Formicidae (1.06) in the third month. During the 2021 off-season, one month after harvesting, Nabidae (1.86), Formicidae (1.16) and Geocoridae (1.06) were the most abundant. At the second month harvesting, Nabidae (1.85), Formicidae (1.78) and Syriphidae (1.25) were the most abundant. In the third month Syriphidae (2.44) and Nabidae (1.81) were the most abundant (Figure 5.4).

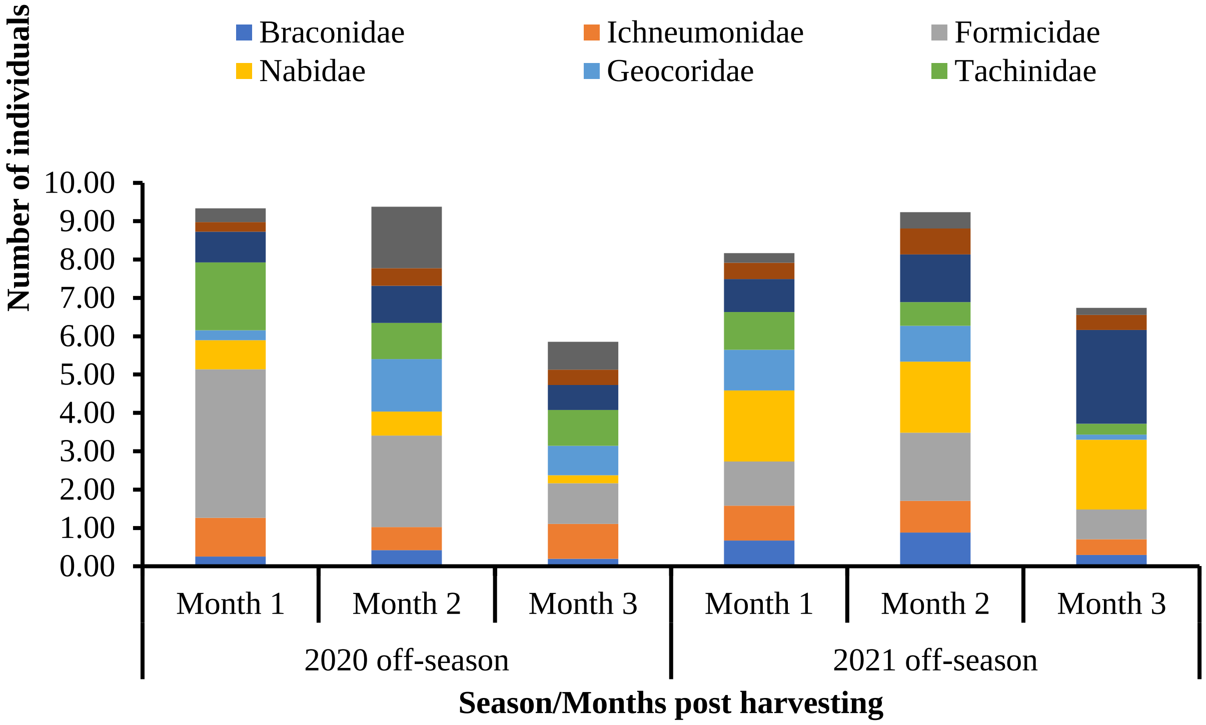

Figure 5. 4: Total abundance of natural enemy families (Mean ± SE) in Rongai months after dolichos harvesting during 2020 and 2021 off-season.

5.5.3 Pearson correlation of field margin plants and natural enemy during off and on cropping seasons

Pearson correlation analysis showed a nonsignificant positive association for margin richness, margin abundance on natural enemy abundance off season ($P>0.05$) (Table 5.6). However, for plant diversity there was a positive significant correlation for natural enemy abundance during the off season ($P=0.002$). Field margin richness had a positive significant association with natural enemy abundance during the season. In addition, field margin richness had a negative signification association with aphid abundance ($P=0.04$) (Table 5.6).

Table 5. 6: Pearson correlation matrix between margin plants, natural enemies, bean aphids and dolichos grain yield.

		FMV Abundance	**FMV Richness**	**FMV Diversity**	**NE Abundance on-season**	**Aphid abundance**
NE Abundance off-season	1	r=0.33 P=0.09	r=0.36 P=0.06	r=0.55 P=0.002	r=0.13 P=0.47	r=-0.09 P=0.58
FMV Abundance		1	r=0.74 P<0.0001	r=0.62 P=0.0005	r=0.44 P=0.01	r=-0.22 P= 0.27
FMV Richness			1	r=0.72 P<0.0001	r=0.69 P<0.0001	r=-0.38 P=0.04
FMV Diversity				1	r=0.35 P=0.06	r=-0.19 P= 0.32
NE Abundance on-season					1	r=-0.49 P=0.004
Aphid abundance						1

NE=Natural enemies; FMV= Field margin vegetation

5.6 Discussion

The results from this study show that field margin habitats offer refuge for natural enemies and other beneficial insects outside the main cropping season. The data further demonstrate that plant species at crop borders may play a key role in supporting natural enemy biodiversity at the beginning of each growing season as the number of natural enemy groups increased in months after harvesting. Field margins plants and semi-natural habitats can serve as biodiversity reservoirs, providing complementary resources and refuges for many natural enemies of pests (Fusser *et al.*, 2017; Scott & Harmon-Threatt, 2021). These non-crop habitats are more often undisturbed, semi-permanent, permanent and or/ regenerative in case the plants are multipurpose compared to crop fields which are frequently manipulated and disturbed (Amoabeng *et al.*, 2020; Holland *et al.*, 2016). Beneficial organisms which include natural enemies (predators and parasitoids) and pollinators require stable habitat with resources (prey, pollen and nectar) and shelter to sustainably deliver on natural pest regulation and pollination (Jado *et al*, 2019). Other studies from agricultural systems have highlighted the importance of continuity of resources to support beneficial insects, i.e. specifically ensuring that resources such as nectar, prey and shelter are available year-round, not just when the crop is growing (Schellhorn *et al*., 2015). Many natural enemies multiply in response to the availability of the food, hence there is a "time lag" between pests and natural enemy fluctuations. Natural enemies are highly mobile; their movement in and out of the crop will depend on how far their refuge places are and availability of prey and food. Therefore, throughout the season there will be fluctuation in population hence the need to assess their dynamics at several crop stages (Zhao *et al*., 2013). It is worth remembering that as plant communities change the associated organism species and population also changes, in this case as the crop stages and margin species change so do the natural enemies and pests and this influences their succession and colonization (Chaplin-Kramer *et al*., 2011).

A majority of work on biocontrol and agroecology has historically focused on temperate regions (Steward *et al.*, 2014). As these regions usually experience a cool or cold winter, this tends to relieve pest pressure, as well as potentially reducing natural enemy populations, for several months of the year. Conversely, in tropical systems such as where lablab is grown in sub-Saharan Africa, even outside the cropping season there is no comparable winter, and the ecology is typically influenced more by rainfall than temperature patterns. As a result, the role of off-season vegetation and refugia may be even more critical than in temperate systems, and yet there is very little research exploring this particular element of the agroecosystem.

A higher number of natural enemies were observed in the second month after crop removal, suggesting the contribution of stable habitat in population build up. This could be an indication that if the habitats were to remain undisturbed to the next crop cycle, relatively high numbers of natural enemies could be observed at the beginning of the crop season (Opatovsky & Lubin, 2012; Fountain, 2022;). Conservation biological control is a key strategy towards sustainable pest management with the main focus on enhancing diversity and populations of naturally occurring predatory and parasitic invertebrate taxa (Balzan *et al*., 2016). The presence of low numbers, species richness and diversity of natural enemy groups at the onset of cropping seasons are less likely to regulate pest population (Rusch e*t al.*, 2010).

Fluorescent dye has been used to demonstrate that natural enemies can move from margin plants into crop fields indicating that non-crop habitat can provide resources or refuge for natural enemies (Elisante *et al.*, 2020; Mkenda *et al.*, 2019). In the same study, results showed that colonisation and population build up was high at the beginning of the season (vegetation stage) as the natural enemies came into the ecosystem. The natural enemy populations reduced as resources started declining at flowering and were very high at the podding stage as resources increased. Here we observed higher natural enemy numbers in the field margins that led to more natural enemy in the crop field which is supported by other studies in crop-non-crop habitat interactions (Bertrand *et al*., 2016), and higher natural enemy numbers in margin plants was corelated with increased pest management (Mkenda *et al*., 2019).

While dolichos during its flowering season may provide nectar and pollen for beneficial insects in its own right (in common with other mass-flowering crops) (Holzschuh *et al*., 2013), outside the cropping season these invertebrate species continue to need food sources and alternative prey and so are reliant on non-crop plants for prey, pollen and nectar, such as from field margins. In a tropical system in particular, plant diversity will support a wider range of flowering phenology leading to continuity in resources where more species are present. Native and perennial plants may offer particular benefits as part of the assemblage (Cahenzli *et al*., 2019; Pfiffner *et al*., 2019). This could be the reason why the natural enemy populations were relatively uniform in abundance even though diverse in composition across the locations.

The composition of the natural enemy communities differed in Njoro and Rongai over the two off-seasons. Generally, flying insects were the most abundant compared to ground dwelling, most likely as a result of the sampling methods used or retained availability and population increase and survival in the habitat. During crop harvesting the habitats are highly disturbed and insect families which detect the changes are bound to rapid changes and dispersal (Opatovsky & Lubin, 2012; Skirvin *et al*., 2011). Field margin plants and semi-natural habitat are the immediate dwelling options for these arthropods (Arnold *et al*., 2021; Mkenda *et al*., 2019). Availability of these non-crop habitats ensures a rapid shift promoting arthropod dispersal from the disturbed crop fields. In farmers' fields that were cleared immediately after harvesting, these clearance activities resulted in a simple habitat which supported only low numbers of natural enemies. This observation is supported by Cloyd (2020) who reported that availability of diverse plant species at the field margins was a clear indicator of higher diversity and abundance of natural enemies at the onset of cropping season. This study showed that farms with higher abundance of non-crop vegetation in the off-season also have higher abundances of natural enemies, which previous work in this region has demonstrated can support pest management and improved yield.

There was a seasonal variation in the abundance and species richness of plants across the study areas. This difference to a greater extent can be related to agricultural practices carried out in these regions. For instance, Njoro region is classified as a high agricultural zone with intense agricultural activities compared to Rongai which had reduced agricultural activities due to prevailing climatic conditions. However, recommending particular plants for field margins can be difficult, as plants vary in their nutritional requirements and seasonality (Lahiru & Costamagna, 2019) and some can be secondary hosts to pests or crop diseases (Buck *et al*., 2023). Furthermore, many plant herbivores are controlled by natural enemies that are habitat generalists, which makes it difficult to identify their requirements (Sorribas *et al*., 2016; Stoddard *et al*., 2010). The inconsistent results perhaps could be attributed to either failure of field margin plants to provide needed resources for the natural enemy community or competitive interactions among generalist predators (Fiedler & Landis, 2007; Ramsden *et al*., 2014; Karp *et al.*, 2018).

Conclusion

Findings from this study demonstrates that populations of agriculturally-relevant natural enemies can be boosted by plant rich field margins outside the cropping period, and therefore that this habitat requires special consideration. In particular, “banking” natural enemies – particularly in warm climates where insect reproduction can take place year-round and beneficial populations can keep building before a cropping season may protect the crop better. Therefore, measures to sustain natural enemies outside the cropping season could allow farms to enter the main cropping season with better baseline populations of natural enemies. The composition of the natural enemy communities shifts across the season, and dissecting the reasons for this and the practical consequences for agriculture will be important in tailoring management for sustainable lablab production in future, especially in the face of changing climate and land-use in these regions.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General discussion

The findings of this study showed that integration of cropping systems, field margin vegetation and use of natural enemies can be effective in the regulation of bean aphid populations. Field margin vegetation provides an abundance of food resources, habitat structure for shrub dwelling natural enemies and reproduction areas for most insects (Nicholls & Altieri, 2013). This study identified different field margin vegetation associated with natural enemy conservation. Their presence reduced the bean aphid population mostly at the margins as compared to the centre of the dolichos crop. Similar patterns were observed by Völkl and Stechmann (1998) who reported that the position of aphid colonies within the field affects the efficiency of the natural enemies.

The two cropping systems showed differences in the diversity of natural enemies and bean aphid populations. The maize-dolichos intercrop had higher diversity index, abundance index, and evenness index than dolichos monocrop. The presence of natural enemies (number and species) in the intercrop was probably influenced by the availability of food, suitable climatic and habitat conditions (Lopes *et al*., 2016; Ratnadass *et al*., 2013; Trenbath, 1993). Intercropped dolichos provided protection against attack by bean aphids and had a lower bean aphid population. Bean aphids are phloem feeders of meristematic leaves and shoots, they have high reproductive ability with short generations and are considered to be host specific. Maize can also produce insect repelling semio-chemicals and the outcome being bottom-up effects (from a lower trophic level to a higher one) against pests of the main crop (Ratnadass *et al*., 2013). In contrast, pests attracted by associated plants (traps) are less likely to wander onto the main crop, whereas natural enemies may be attracted to the crop and help regulate the pest population. These results are in accordance with studies by Khan *et al.* (2008, 2018) and Okoko (2001).

Pan trapping, sweep netting and use of sticky traps were used to capture the natural enemies of bean aphids. These methods are widely used for assessing the abundance and diversity due to affordability and efficacy in trapping species that are easily attracted to the traps and easily identified. Pan trapping method targeted to capture crawling natural enemies inside the crop and at the field margin, sweepnetting targeted to capture the flying natural enemies while the sticky traps were used to monitor movement of natural enemies from the field margins to the centre of the crop. The beneficial natural enemy families that were captured were Hymenopteran, Diptera, Coleoptera, Arenae and Orthopteran while the pests were Hemiptera and Homoptera and the non-targets were from Lepidoptera families. The most abundant species were from the Hymenoptera family. This family mostly consists of the natural enemies of bean aphids and they have a variety of food sources which are not limited. The relative abundance of the natural enemies inside the plots and on the field margin vegetation at different cropping systems were different. The results are similar to Balzan and Moonen (2014) who reported non crop habitats maintained high insect diversity at the borders as compared to the crop centre.

Increasing landscape diversity or complexity can increase the abundance of parasitoids and parasitism rates for *Aphidius colemani*, although this may not be the case for all parasitoid species and likely depends on their differing resource requirements. Our study identified Aphidius colemani and *Lysiphlebus testaceipes* to be the most abundant bean aphid primary parasitoids in Kenya, hence they should be the target of conservation biological control in the smallholder agro-ecosystems. However, it would be important to take into account the effect of secondary parasitoid and pest species, so that they do not also benefit from these interventions.

Rongai location had the most abundant floral sources during the off-season cropping period. This difference is due to the agricultural practices on the two locations. Field margin vegetation left to grow during off-cropping season provides resources for the natural enemies to pupate during low periods of food sources (Olson & Wäckers, 2007). Dolichos yields were high in monocrop as compared to intercrop systems. However, the bean aphid damage was high in the monocrop system. Yield aspect is a combination of many factors which are related hence yield reduction in the intercropped dolichos cannot be attributed to the aspect of pest damage. However, the intercrop system has a relative yield advantage from the maize and dolichos.

Insect diversity and abundance can be increased by raising the diversity and area of field margin vegetation. The specific management of particular insect species requires more detailed knowledge of their habitat requirements. Other studies of field margin populations that can be explored are the examination of diversity, effect of structural features on overwintering densities, specific species of field margin vegetation that can be used in the hedges of crops which can provide habitat to the arthropods.

6.2 Conclusions

1. Diverse field margin vegetation provides a habitat that supports diverse natural enemies of bean aphids with *Aphidius colemani* as the most abundant parasitoid within the Njoro and Rongai farmlands. Besides, intercropping dolichos with maize has the potential of maintaining low populations of bean aphids. If conserved the natural enemies can play a major role in bean aphid natural pest regulation. The presence of field margin vegetation around crop borders potentially increased dolichos grain yields thought to have been due to increased natural enemies and aphid control.
2. Two field margin species, *Galinsoga parviflora* and *Bidens pilosa,* have demonstrated good potential to support the survival of natural enemies of bean aphids like the tachinidae and *Aphidius colemani*. Allowing such field margin species to grow around crop borders has the potential of enhancing populations of natural enemies by providing nectar and pollen resources.
3. Field margin plants available outside the main crop growing season offer floral resources that support the continued survival of natural enemies of bean aphids between cropping seasons.

6.3 Recommendations

The following are recommendations derived from this study:

1. Farmers are encouraged to adopt intercropping and conservation of diverse field margin vegetation around croplands to support natural enemy populations for enhanced natural pest regulation and to promote increased crop yields.
2. Conserved or planted common weed species like *Bidens pilosa* and *Galinsoga parviflora,* are promoted as preferred field margin vegetation for enhanced natural enemy promotion due to their bright coloured petals that attract nectar and pollen feeding natural enemies of bean aphids.
3. After crop harvest, clearing of vegetation around croplands should be delayed to a few days to the new season in order to support sustained provision of floral resources to feed bean aphid natural enemies. Alternatively, farmers are encouraged to adopt planted field margin vegetation using recommended common weed species.

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APPENDICES

Appendix A: Mean monthly rainfall (mm) and temperature (ºC) for Njoro and Rongai in the year 2019 and 2020.

Month	**Njoro**			**Rongai**		
	Rainfall	**Temperature**		**Rainfall**	**Temperature**	
		Max	**Min**		**Max**	**Min**
January	21.0	24.7	13.9	43.98	28.7	15.1
February	21.0	26.6	14.5	36.62	29.7	16.5
March	53.3	27	15.5	48.97	29.9	15.2
April	109.9	26.9	15.9	84.97	28.1	14.5
May	74.4	24.7	15.5	75.52	25.7	13.1
June	53.4	22.4	14.7	40.08	24.0	13.0
July	61.7	22.2	13.5	50.38	25.6	13.9
August	78.4	22.5	13.1	86.14	24.2	14.4
September	53.7	24.7	13.3	50.17	22.0	14.5
October	94.6	22.9	13.1	92.61	25.4	15.8
November	114.8	22.8	14.4	104.43	25.2	16.0
December	45.6	21.1	14.3	75.18	25.8	16.4

Appendix B: Analysis of variance for species richness, total abundance and Simpson diversity index (SDI) of field margin plants in Njoro and Rongai farmers filed during 2019 and 2020 cropping seasons.

Source of variation	df	Species Richness	Total Abundance	Simpson diversity index (SDI)
Location	1	0.005	0.113	1.106
		0.945	0.737	0.296
Season	1	0.798	0.034	2.426
		0.374	0.854	0.123
Farmer (Replicate)	7	1.139	0.107	1.240
		0.348	0.998	0.291
Treatment	1	0.383	0.168	0.028
		0.538	0.683	0.867
Growth habitat	1	88.678	21.116	54.429
		<0.0001	<0.0001	<0.0001
Location×Season	1	1.063	0.066	0.632
		0.306	0.797	0.429
Location×Farmer	1	0.933	0.121	1.583
		0.486	0.997	0.152
Location×Treatment	1	0.005	0.168	0.114
		0.945	0.683	0.737
Location×Growth habitat	1	5.788	19.907	1.450
		0.018	<0.0001	0.232
Season×Farmer	7	0.151	0.133	0.286
		0.993	0.996	0.958
Season×Treatment	1	0.005	0.030	0.142
		0.945	0.863	0.707
Season×Growth habitat	1	0.005	4.889	3.321
		0.945	0.030	0.072

Appendix B: Continued…

Source of variation	**df**	**Species Richness**	**Total Abundance**	**Simpson diversity index (SDI)**
Location×Season×Farmer	7	1.797	0.125	2.023
		0.099	0.996	0.062
Location×Season×Treatment	1	2.084	0.049	0.074
		0.153	0.825	0.786
Location×Season×Growth habitat	1	0.005	0.013	`0.058
		0.945	0.911	0.810
Location×Farmer×Treatment	7	0.562	0.175	0.509
		0.785	0.990	0.825
R^2		0.618	0.387	0.560
F		2.845	1.113	2.245
Pr > F		<0.0001	0.333	0.001

F= f-value, Pr>F= P value

Appendix C: ANOVA for species richness, abundance, species diversity and species evenness of invertebrate taxa in Njoro and Rongai farmers' fields during 2019 and 2020 cropping seasons.

Source of variation	**df**	**Species richness**	**Species abundance**	**Diversity index**	**Evenness**
Trapping method (TM)	2	75.970	123.458	63.016	0.955
		<0.0001	<0.0001	<0.0001	0.391
Season (S)	1	69.334	10.670	870.638	155.493
		<0.0001	0.002	<0.0001	<0.0001
Location (L)	1	5.364	0.575	1.319	2.393
		0.024	0.451	0.256	0.127
Crop stage (CS)	3	1.781	9.920	0.374	0.242
		0.161	<0.0001	0.772	0.867
Trapping area (TA)	1	0.799	15.474	0.210	0.210
		0.375	0.000	0.648	0.649
Season×Location	1	9.867	0.110	0.966	1.465
		0.003	0.741	0.330	0.231
Season×Trapping area	3	0.497	10.981	1.536	1.774
		0.484	0.002	0.220	0.188
Location×Trapping area	3	0.073	0.180	0.002	0.314
		0.788	0.673	0.966	0.578
Trapping method×Season×Location	3	3.354	3.685	4.040	1.438
		0.015	0.010	0.006	0.233
Trapping method×Season×Crop stage		1.685	9.823	2.959	1.094
	6	0.094	<0.0001	0.003	0.382
TM×S×L×CS×TA	6	2.121	7.493	0.802	1.296
		0.064	<0.0001	0.572	0.274
R^2		0.860	0.905	0.953	0.785
F		14.269	22.097	47.031	8.466
Pr > F		<0.0001	<0.0001	<0.0001	<0.0001

F= f-value, Pr>F= P value.

Appendix D: Analysis of variance for bean aphid damage severity, abundance and incidence in Njoro and Rongai farmer's field during 2019 and 2020 cropping seasons.

Source of variation	**df**		**Aphid severity**	**Aphid abundance**	**Aphid incidence**
Season	1	F	18.08	131.50	227.23
		Pr > F	<0.0001	<0.0001	<0.0001
Location	1	F	7.71	1.15	11.72
		Pr > F	0.006	0.285	0.001
Crop growth stage	4	F	29.55	23.91	103.179
		Pr > F	<0.0001	<0.0001	<0.0001
Cropping system	1	F	58.09	46.88	158.11
		Pr > F	<0.0001	<0.0001	<0.0001
Farmer	7	F	1.80	1.56	1.34
		Pr > F	0.086	0.145	0.231
Season×Location	1	F	0.48	4.21	0.18
		Pr > F	0.485	0.041	0.666
Season×Crop growth stage	7	F	7.21	3.56	15.28
		Pr > F	0.000	0.015	<0.0001
Season×Cropping system	1	F	9.36	54.41	83.04
		Pr > F	0.002	<0.0001	<0.0001
Location×Crop growth stage	4	F	4.20	3.54	2.97
		Pr > F	0.003	0.008	0.020
Location×Cropping system	1	F	0.87	0.65	0.76
		Pr > F	0.349	0.420	0.382
Crop growth stage×Cropping system	4	F	3.57	10.42	25.63
		Pr > F	0.007	<0.0001	<0.0001
Season×Location×Crop growth stage	4	F	2.96	2.24	0.41
		Pr > F	0.033	0.083	0.739

Appendix D: Continued…

Source of variation	df		Aphid severity	Aphid abundance	Aphid incidence
Season×Location×Cropping system	1	F	0.22	2.11	5.61
		Pr > F	0.634	0.147	0.019
Season×Crop growth stage×Cropping system	4	F	2.86	3.47	4.65
		Pr > F	0.037	0.017	0.003
Location×Crop growth stage×Cropping system	4	F	0.89	0.81	0.29
		Pr > F	0.467	0.518	0.881
Season×Location×Crop growth stage×Cropping system	4	F	2.48	1.57	0.69
		Pr > F	0.062	0.197	0.553
R²			0.55	0.61	0.80
F			7.21	9.47	23.78
Pr > F			<0.0001	<0.0001	<0.0001

F= f-value, Pr>F= P value

Appendix E: Analysis of variance for dolichos bean grain yield in Njoro and Rongai farmer's field during 2019 and 2020 cropping seasons.

Source of variation	**df**		**Grain yield tons/ha**
Season	1	F	0.52
		Pr > F	0.48
Location	1	F	0.42
		Pr > F	0.52
Treatment	1	F	8.79
		Pr > F	0.00
Replicate	7	F	0.51
		Pr > F	0.82
Season × Location	1	F	0.64
		Pr > F	0.43
Season×Treatment	1	F	0.26
		Pr > F	0.62
Location×Treatment	1	F	0.40
		Pr > F	0.53
Season×Location×Treatment	1	F	0.49
		Pr > F	0.49
R^2			0.27
F			1.14
Pr > F			0.36

F= f-value, Pr>F= P value

Appendix F: Analysis of variance for overall abundance of natural enemies captured in Njoro and Rongai during 2020 and 2021 off seasons.

Source of variation	DF	F-value	Pr > F
Season	1	1.26	0.262
Location	1	1.79	0.181
Months after harvest	2	8.11	0.000**
Rep	7	1.47	0.174
Season ×Location	1	0.00	0.960
Season × Months after harvest	2	0.10	0.906
Location × Months after harvest	2	0.25	0.778
Season × Location × Months after harvest	2	0.10	0.905

F= f-value, Pr>F= P value

Appendix G: NACOSTI Research Permit

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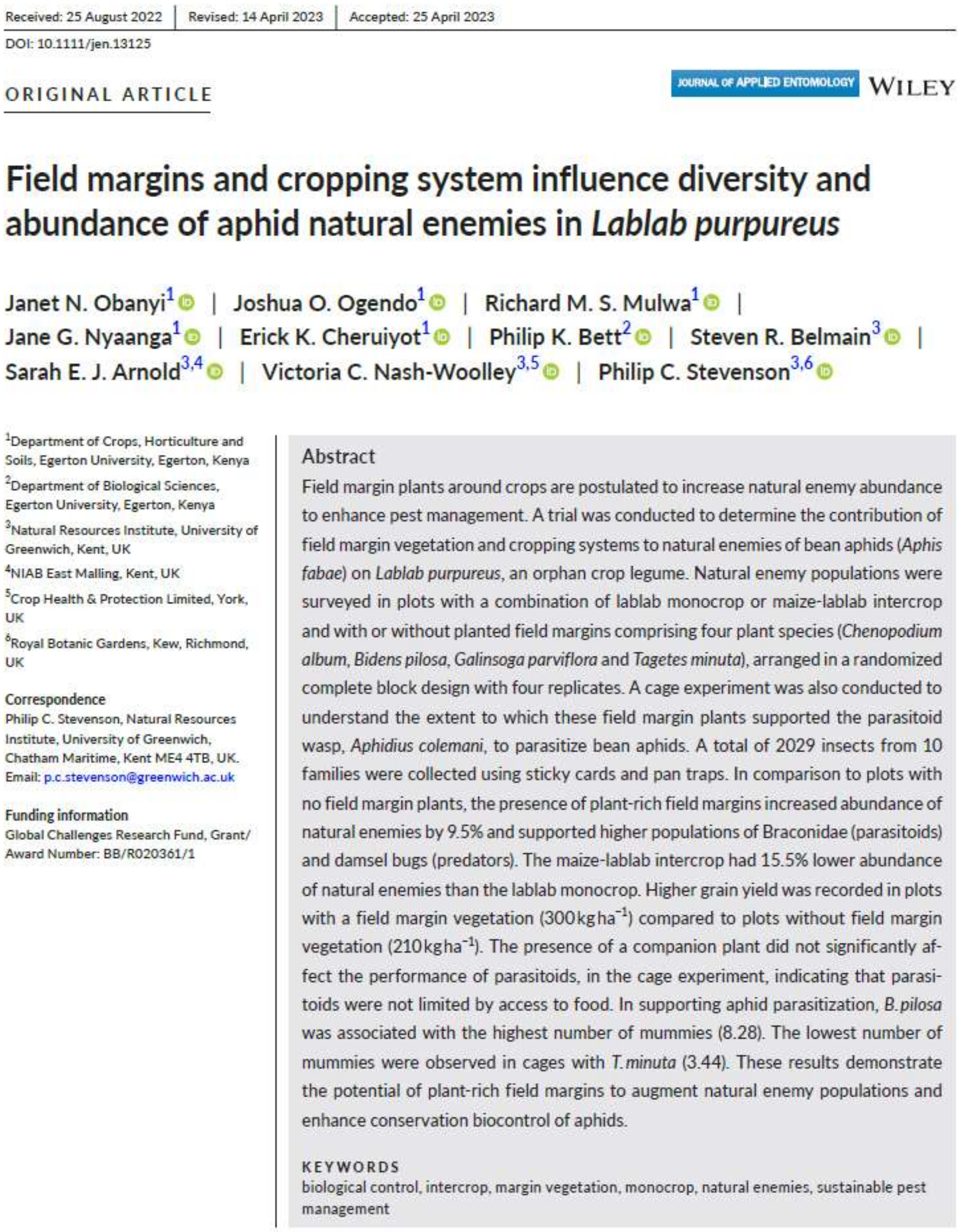
Appendix H: Author's Own Publications and scientific conference presentations

a) Publications in Refereed Scientific Journals

i. Obanyi, J. N., Ogendo, J. O., Mulwa, R. M., Nyaanga, J. G., Cheruiyot, E. K., Bett, P. K., ... & Stevenson, P. C. (2023). Field margins and cropping system influence diversity and abundance of aphid natural enemies in Lablab purpureus. *Journal of Applied Entomology*. https://doi.org/10.1111/jen.13125



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ORIGINAL ARTICLE

JOURNAL OF APPLIED ENTOMOLOGY WILEY

Field margins and cropping system influence diversity and abundance of aphid natural enemies in *Lablab purpureus*

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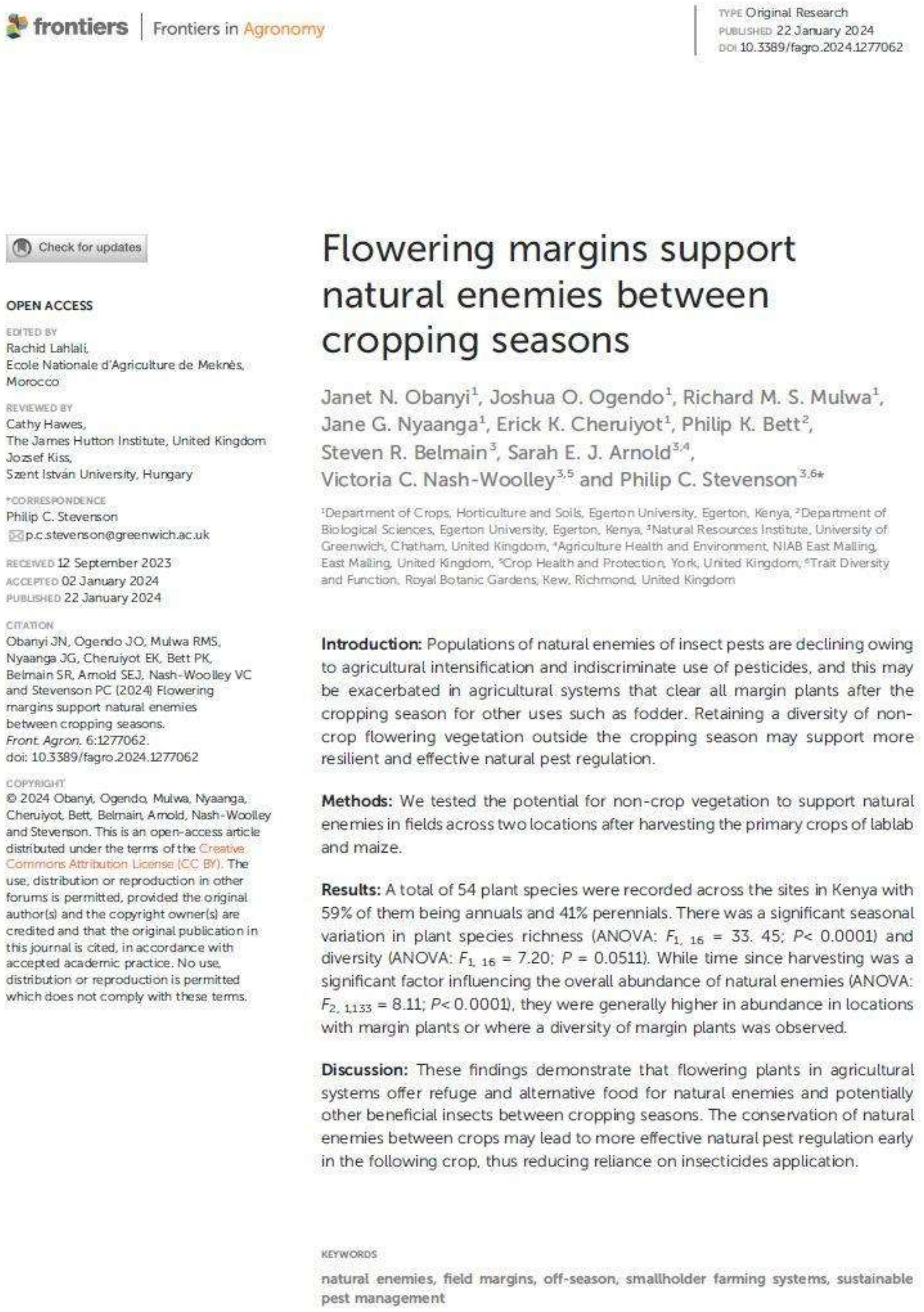
Abstract

Field margin plants around crops are postulated to increase natural enemy abundance to enhance pest management. A trial was conducted to determine the contribution of field margin vegetation and cropping systems to natural enemies of bean aphids (*Aphis fabae*) on *Lablab purpureus*, an orphan crop legume. Natural enemy populations were surveyed in plots with a combination of lablab monocrop or maize-lablab intercrop and with or without planted field margins comprising four plant species (*Chenopodium album*, *Bidens pilosa*, *Galinsoga parviflora* and *Tagetes minuta*), arranged in a randomized complete block design with four replicates. A cage experiment was also conducted to understand the extent to which these field margin plants supported the parasitoid wasp, *Aphidius colemani*, to parasitize bean aphids. A total of 2029 insects from 10 families were collected using sticky cards and pan traps. In comparison to plots with no field margin plants, the presence of plant-rich field margins increased abundance of natural enemies by 9.5% and supported higher populations of Braconidae (parasitoids) and damsel bugs (predators). The maize-lablab intercrop had 15.5% lower abundance of natural enemies than the lablab monocrop. Higher grain yield was recorded in plots with a field margin vegetation (300 kg ha^{-1}) compared to plots without field margin vegetation (210 kg ha^{-1}). The presence of a companion plant did not significantly affect the performance of parasitoids, in the cage experiment, indicating that parasitoids were not limited by access to food. In supporting aphid parasitization, *B. pilosa* was associated with the highest number of mummies (8.28). The lowest number of mummies were observed in cages with *T. minuta* (3.44). These results demonstrate the potential of plant-rich field margins to augment natural enemy populations and enhance conservation biocontrol of aphids.

KEYWORDS
biological control, intercrop, margin vegetation, monocrop, natural enemies, sustainable pest management

ii. **Obanyi *et al*., (2024).** Flowering margins support natural enemies between cropping seasons. Frontiers in Agronomy, 6:1277062. doi: 10.3389/fagro.2024.1277062



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Flowering margins support natural enemies between cropping seasons

Janet N. Obanyi[1], Joshua O. Ogendo[1], Richard M. S. Mulwa[1], Jane G. Nyaanga[1], Erick K. Cheruiyot[1], Philip K. Bett[2], Steven R. Belmain[3], Sarah E. J. Arnold[3,4], Victoria C. Nash-Woolley[3,5] and Philip C. Stevenson[3,6*]

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Introduction: Populations of natural enemies of insect pests are declining owing to agricultural intensification and indiscriminate use of pesticides, and this may be exacerbated in agricultural systems that clear all margin plants after the cropping season for other uses such as fodder. Retaining a diversity of non-crop flowering vegetation outside the cropping season may support more resilient and effective natural pest regulation.

Methods: We tested the potential for non-crop vegetation to support natural enemies in fields across two locations after harvesting the primary crops of lablab and maize.

Results: A total of 54 plant species were recorded across the sites in Kenya with 59% of them being annuals and 41% perennials. There was a significant seasonal variation in plant species richness (ANOVA: $F_{1,\ 16}$ = 33. 45; $P< 0.0001$) and diversity (ANOVA: $F_{1,\ 16}$ = 7.20; P = 0.0511). While time since harvesting was a significant factor influencing the overall abundance of natural enemies (ANOVA: $F_{2,\ 1,133}$ = 8.11; $P< 0.0001$), they were generally higher in abundance in locations with margin plants or where a diversity of margin plants was observed.

Discussion: These findings demonstrate that flowering plants in agricultural systems offer refuge and alternative food for natural enemies and potentially other beneficial insects between cropping seasons. The conservation of natural enemies between crops may lead to more effective natural pest regulation early in the following crop, thus reducing reliance on insecticides application.

KEYWORDS
natural enemies, field margins, off-season, smallholder farming systems, sustainable pest management

b) Conference presentations

i. **Obanyi *et al*. (2023).** Effect of field margin vegetation on natural pest regulation in Dolichos bean (*Lablab purpureus* L.). 1st East Africa Agroecology Conference. March 21-24, 2023, Safari Park Hotel, Nairobi, Kenya.

Transforming Food Systems for Responsible Production, Consumption and Social Wellbeing

Effect of field margin vegetation on natural pest regulation in Dolichos bean (Lablab purpureus L.)

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Introduction

Dolichos bean, *Lablab purpureus*, is an important legume crop grown for human consumption (green and dry seed) and whole plant is used as fodder for cattle (Soetan and Fafunso 2010). The crop has potential to support food security in Africa due to its ability to tolerate drought and salinity (Minde, Venkataramana, & Matemu, 2021). However, its role in a sustainable agricultural system and particularly pest management is less under-stood (Cullis and Kunert 2017). The crop experiences pest infestation by up to 55 species of insects and mites (Govindan, 1974). Aphids are among the most severe invertebrate pests that cause high economic losses (Abate *et al.* 2000; Thejaswi *et al*. 2007; Nahashon *et al.* 2016). Non-crop vegetation around farmlands have been documented to harbor diverse invertebrate taxa composed of pests and beneficial insects (natural enemies, pollinators and nutrient-cyclers) that are valuable in enhancing biological ecosystem services (Balmer et al., 2013). Both populations are usually supported by crops and field margin vegetation but little is known on the importance of field margins in supporting natural enemies of insect pests in tropical agriculture (Bianchi, Booij, & Tscharntke, 2006).

Methodology

The study documented the bean aphid natural enemy populations and potential contributions to enhanced insect pest regulation on Dolichos bean in smallholder farms. Field experiments were conducted during May-December 2019 and March -November 2020 crop growing seasons. Each experiment was laid out in a ran-domized complete block design (RCBD) with eight replications per location. Dolichos monocrop and maize-dolichos intercrop were established on plots measuring 10 m x 10 m with or without 2m planted field margin vegetation perimeter strip. Equal mixed ratio by weight planted field margins comprised of four plant species (*Chenopodium album*, *Bidens pilosa*, *Galinsoga parviflora* and *Tagetes minuta*). Aphid natural enemy populations were collected using sticky cards, pan traps and sentinel plants placed at 5m away from the planted field margin strip and into the crop. Trap placement was at done at three growth stages of the lablab bean crop (seedling vegetative and podding).

Results & Discussion

A total of 2029 insects from 10 families were recorded. Presence of plant-rich field margins increased the abun-dance of natural enemies by (9.5 %) and supported higher populations of Braconidae (parasitoids) and damsel bugs (predators). The maize-dolichos intercrop had 15.5% lower abundance of natural enemies than dolichos monocrop. Higher grain yield was recorded in plots with field margin vegetation (300 kg ha^{-1}) compared to plots without field margin vegetation (210 kg ha^{-1}). For DNA barcoded parasitoids emerged from *Aphis fabae* infested sentinel plants, three primary parasitoids were identified: *Aphidius* sp., *Aphidius colemani*, *Lysiphle-bus fabarum* and *Lysiphlebus* sp. Sentinel plants deployed in high elevation significantly ($P<0.001$) recorded more parasitoids. These results demonstrate that presence of plant-rich field margins contributes to enhanced ecological conservation of natural enemies and biological pest regulation services.

Conclusion

Inclusion of field margin vegetation around farmlands leads to enhanced conservation of invertebrate taxa diversity and abundance, an important functional mechanism of integrated natural pest regulation. However, it is crucial to study the biology of the diverse vegetation and interactions with pests and natural enemies for an optimized ecologically sound biological pest regulation.

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ii. **Obanyi *et al*. (2020).** Diversity and Abundance of Invertebrate Taxa in Smallholder Farms in Nakuru County. Proc. 13th Egerton University Virtual Conference, 24th – 26th November 2020.

Diversity and Abundance of Invertebrate Taxa in Smallholder Farms in Nakuru County: Effect of Dolichos-Based Cropping System and Field Margin Vegetation

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Biological control by use of natural enemies is an emerging eco-friendly pest management method which can provide a sustainable alternative option of controlling pest. However, the conservation of these natural enemies within agricultural production systems is a challenge. Field studies were conducted in smallholder farms in Nakuru County to determine the effect of field margin vegetation in supporting the diversity and abundance of invertebrate taxa. Two cropping systems, dolichos monocrop and maize-dolichos intercrop, were planted in farmers' fields with or without field margin vegetation in Njoro and Rongai sub-counties of Nakuru County during the long rains of 2019 and 2020. The treatments were arranged in randomized complete block design (RCBD) with eight (8) replicates per locality. The abundance and diversity of field margin vegetation in each farm, was determined by throwing a 1 m^2 quadrat thrice per field margin and counting all plant species present and converting them to percent abundance. Invertebrate taxa were trapped using yellow sticky cards, pan traps and sweep netting three times during the cropping season. Trappings were grouped, counted and morphologically identified using taxonomic keys. All data on counts were transformed using square root transformation ($\sqrt{X}+ 0.5$) before being subjected to analysis of variance using PROC GLM in SAS software and treatment means separated using Tukey's HSD test at $P \leq 0.05$. Results showed that there were a total of eighteen (18) perennial species; 8 in Njoro and 10 in Rongai. There were more weeds species in Rongai (17%) compared to Njoro (14%) and more perennial weeds (21%) compared to annual weeds (13%). Mean abundance of invertebrate taxa was higher in Rongai (7.2) farms compared to Njoro (6.8) farms. Across the two environments the invertebrate taxa collected were composed of pests and their natural enemies and other insects like flies. The results further showed that invertebrate taxa were significantly higher in the Dolichos monocrop (3.6) than in the maize-dolichos intercrop (2.2). These results show that inclusion of field margin vegetation around smallholder farms enhances the conservation of diverse invertebrate taxa which is an important functional mechanism of managing different pests in integrated pest management.

Keywords: biological control, conservation, invertebrate taxa, natural enemies, plant diversity