

**EFFECTS OF BOTANICAL INSECTICIDES AND FIELD MARGIN VEGETATION
ON APHIDS AND THEIR NATURAL ENEMIES IN DOLICHOS BEAN (*Lablab
purpureus* L.) PRODUCTIVITY**

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**A Thesis Submitted to the Graduate School in Partial Fulfillment of the Requirements
for the Master of Science Degree in Crop Protection of Egerton University**

EGERTON UNIVERSITY

JULY 2022

DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been submitted for examination in any institution.

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DEDICATION

This thesis is dedicated to my uncle, John Okello who taught me to work hard, my brother Francis Omondi who was very supportive and to God who gave me a good health to achieve this dream.

ACKNOWLEDGEMENTS

I thank Egerton University for institutional support and the provision of field space to conduct experiments. I am also grateful to the Natural Pest Regulation on Orphan Crop Legumes in Africa (NaPROCLA) Project, through the Principal Investigator, Prof. Joshua O. Ogendo, for financial support of this study. I also wish to thank my supervisors Prof. Joshua Ogendo and Dr. Philip K. Bett and the other NaPROCLA scientists, Dr. Jane G. Nyaanga, Prof Eric K. Cheruiyot and Prof Richard M.S. Mulwa for their professional advice and guidance throughout the study period. Finally, I wish to acknowledge my late parents, fellow NaPROCLA-sponsored students, sisters, brothers and friends for their encouragement, moral and spiritual support throughout the study period.

ABSTRACT

The black bean aphid (*Aphis fabae* S.) is a major insect pest that causes significant yield losses in the production of legumes such as dolichos bean (*Lablab purpureus* L.). Synthetic insecticides reduce insect pest damage while ignoring the declining biodiversity of natural enemies such as predators and parasitoids. A field and cage exclusion experiments were conducted in a randomized complete block design (RCBD) to determine the effectiveness of selected commercial botanical insecticides and field margin vegetation (FMV) in the management of bean aphids as well as the abundance of natural enemies in dolichos bean. The treatment comprised of three botanical insecticides: Pyerin75EC[®] (1% natural pyrethrin + 1% neem oil + 25% garlic extracts), Nimbecidine[®] (0.03% Azadirachtin + 90.57% neem oil) and Pyeneem 20EC[®] (1% natural pyrethrin + 1% neem oil) tested alongside synthetic insecticide Duduthrin 1.75EC[®] (1.75% *lambda*-cyhalothrin) and untreated as positive and negative controls, respectively. The effect of these treatments was evaluated in plots where the dolichos crop was either surrounded by FMV containing weed species black jack (*Bidens Pilosa* L.), Mexican marigold (*Tagetes minuta* L.), goat weed (*Ageratum conyzoides* L.) and gallant soldier (*Galinsoga parviflora* Cav.) or with no FMV. The botanical insecticides and positive control (Duduthrin) were applied at the second trifoliate and sixth trifoliate dolichos growth stages. Data collected on aphid abundance, damage severity, and percent incidence and natural enemy population as well as yield and yield components of dolichos were subjected to analysis of variance using Statistical Analysis System version 9.2. Results showed that botanical and synthetic insecticides reduced aphid abundance by 88% and 80%, respectively compared to the untreated control. In plots surrounded with FMV and treated with botanical insecticides, a high number of natural enemies (33.55%) were collected as compared to no FMV (24.88%). High grain yields of 2.55-3.04 and 2.95-3.23 tons/ha were observed for both botanical insecticides and synthetic insecticides in the presence of FMV as compared to no FMV 1.86-2.79 and 2.55-2.85 tons/ha, respectively. In the cage experiment, results revealed that integration of field margin vegetation and botanical insecticides had a higher recovery of ladybird beetles compared application of the botanical insecticides without the FMV. Botanical insecticides resulted in a higher number of ladybird beetles in presence of FMV (3.91-4.43) as compared to no FMV (3.16-3.67). The results demonstrated that commercial botanical insecticides in combination with field margin vegetation are viable alternatives to synthetic insecticides in the control of insect pest.

TABLE OF CONTENTS

DECLARATION AND RECOMMENDATION	ii
COPYRIGHT.....	iii
DEDICATION.....	iv
ACKNOWLEDGEMENTS	v
ABSTRACT.....	vi
LIST OF FIGURES	xi
LIST OF TABLES	xii
LIST OF ABBREVIATIONS AND ACRONYMS	xiv
CHAPTER ONE	1
INTRODUCTION.....	1
1.1 Background information	1
1.2 Statement of the problem	2
1.3 Objectives	3
1.3.1 Broad objective.....	3
1.3.2 Specific objectives.....	3
1.4 Hypotheses.....	3
1.5 Justification of the study	4
CHAPTER TWO	5
LITERATURE REVIEW	5
2.1 Origin and botany of Dolichos bean	5
2.2 Climatic requirements for Dolichos bean production.....	5
2.3 Production and utilization of Dolichos bean.....	6
2.4 Constraints to production and yield of Dolichos bean.....	7
2.5 Distribution and biology of bean aphid (<i>Aphis fabae</i> S.).....	8
2.6 Nature and extent of damage caused by bean aphid	9
2.7 Control methods of bean aphids	9
2.7.1 Cultural control.....	10
2.7.2 Synthetic insecticides control.....	10
2.7.3 Botanical insecticides control.....	11
2.7.4 Biological control	12
2.8 Conservation of natural enemies.....	13

CHAPTER THREE	14
EFFECT OF BOTANICAL INSECTICIDES AND FIELD MARGIN VEGETATION ON BEAN APHIDS AND NATURAL ENEMY POPULATIONS IN DOLICHOS BEAN (<i>Lablab purpureus</i> L.).....	14
Abstract.....	14
3.1 Introduction.....	14
3.2 Materials and methods	15
3.2.1 Experimental site	15
3.2.2 Experimental procedure	16
3.2.3 Data collection.....	18
3.2.4 Data analyses	22
3.3 Results.....	23
3.3.1 Effect of cropping cycle and field margin vegetation on aphid abundance, damage severity and percent incidence	23
3.3.2 Effect of botanical insecticides on aphids' abundance.....	24
3.3.3 Effect of botanical insecticides on damage severity	28
3.3.4 Effect of botanical insecticides on aphid incidence	32
3.3.5 Effect of cropping cycle and field margin vegetation on the abundance of natural enemies (parasitic wasps, tachinid flies and ladybird beetles).	36
3.3.6 Effect of botanical insecticides on the abundance of parasitic wasps	39
3.3.7 Effect of botanical insecticides on the abundance of ladybird beetles.....	42
3.3.8 Effect of botanical insecticides on the abundance of tachinid flies	45
3.4 Discussion	48
 CHAPTER FOUR.....	 51
EFFECT OF BOTANICAL INSECTICIDES AND FIELD MARGIN VEGETATION ON GROWTH AND YIELD OF DOLICHOS BEAN (<i>Lablab purpureus</i> L.)	51
Abstract.....	51
4.1 Introduction.....	51
4.2 Materials and methods	53
4.2.1 Experimental site and procedure	53
4.2.2 Data collection.....	53
4.2.3 Data analyses	53
4.3 Results.....	54

4.3.1 Effect of cropping cycle and field margin vegetation on undamaged pods, damaged pods, seeds per pod, 100-seed weight, grain yield and above-ground biomass	54
4.3.2 Effect of botanical insecticides on undamaged pods, damaged pods, seeds per pod, 100-seed weight, grain yield and above-ground biomass	56
4.4 Discussion	59
 CHAPTER FIVE	61
EFFECT OF BOTANICAL INSECTICIDES AND FIELD MARGIN VEGETATION ON RECOVERY OF LADYBIRD BEETLES (<i>Chelomenes lunata</i>, COLEOPTERA: COCCINELLIDAE) IN DOLICHOS BEAN.....	61
Abstract.....	61
5.1 Introduction.....	61
5.2 Materials and methods	62
5.2.1 Rearing of black bean aphids and predators (Ladybird beetles- <i>Chelomenes lunata</i>)	62
5.2.2 Experimental procedure	63
5.2.3 Data collection.....	64
5.2.4 Data analyses	65
5.3 Results.....	66
5.3.1 Analysis of variance for number, percent change of population density and percent reduction of ladybird beetles	66
5.3.2 Efficiency of the selected botanical insecticides and field margin vegetation on the recovery of ladybird beetles	67
5.4 Discussion	73
 CHAPTER SIX	75
GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS	75
6.1 General discussion	75
6.2 Conclusions.....	76
6.3 Recommendations.....	76
 REFERENCES.....	77
APPENDICES	93
Appendix A: Analysis of variance for the aphid abundance, damage severity and percent incidence on dolichos bean.	93

Appendix B: Combined mean comparison for aphid abundance, damage severity and percent incidence on dolichos bean as influenced by selected botanical insecticides and field margin vegetation.....	94
Appendix C: Analysis of variance for the abundance of key natural enemy species found on dolichos bean.	95
Appendix D: Combined mean comparison for the abundance of parasitic wasps, tachinid flies and ladybird beetles as affected by selected botanical insecticides and field margin vegetation	96
Appendix E: Analysis of variance for growth and yield components in dolichos bean.	97
Appendix F: Combined mean comparison for undamaged pods/plant, damaged pod/plant, seeds/pod, 100seedweight, grain yield, biomass and plant height as influenced by selected botanical insecticides and field margin vegetation	98
Appendix G: Dolichos bean yield from botanical insecticides, Duduthrin and untreated when cropped with a field margin vegetation present or absent.	100
Appendix H: Analysis of variance for population densities of ladybird beetles on dolichos bean as influenced by botanical insecticides and field margin vegetation.	101
Appendix I: Combined mean comparison for population densities of ladybird beetles on dolichos bean as influenced by botanical insecticides in the presence of field margin vegetation.	102
Appendix J: Author's publications	103
Appendix K: Research permit.....	105

LIST OF FIGURES

Figure 2. 1: Black bean aphids (<i>Aphis fabae</i> S.) on dolichos at: (A) early vegetative stage and (A) podding stage	9
Figure 3. 1: Plots with field margin vegetation (FMV) (A) and without field margin vegetation (B) at Egerton University, Field 8	17
Figure 3. 2: Yellow pan traps and yellow sticky traps used for natural enemies sampling at: (A) crop centre and (B) field margin vegetation.....	22
Figure 3. 3: Collected natural enemies of bean aphid: Parasitic wasps (A), Tachinid flies (B) and ladybird beetles (C)	38
Figure 3. 4: Abundance (Mean $\pm$ SE; n=8) of parasitic wasps in dolichos bean pre-and post-botanical insecticides application in the presence or absence of field margin vegetation (May-December 2019cropping cycle).	40
Figure 3. 5: Abundance (Mean $\pm$ SE; n=8) of parasitic wasps in dolichos bean pre-and post-botanical insecticides application in the presence or absence of field margin vegetation (March-Novemeber 2020 cropping cycle).	41
Figure 3. 6: Abundance (Mean $\pm$ SE; n=8) of ladybird beetles in dolichos bean pre-and post-botanical insecticides application in the presence or absence of field margin vegetation (May-December 2019cropping cycle).	43
Figure 3. 7: Abundance (Mean $\pm$ SE; n=8) of ladybird beetles in dolichos bean pre-and post-botanical insecticides application in the presence or absence of field margin vegetation (March-November 2020 cropping cycle).	44
Figure 3. 8: Abundance (Mean $\pm$ SE; n=8) of tachinid flies in dolichos bean pre-and post-botanical insecticides application in the presence or absence of field margin vegetation (May-December 2019cropping cycle).	46
Figure 3. 9: Abundance (Mean $\pm$ SE; n=8) of tachinid flies in dolichos bean pre-and post-botanical insecticides application in the presence or absence of field margin vegetation (March-November 2020 cropping cycle).	47
Figure 5. 1: Cage experiment layout: sample cage experimental layout (A), cage without field margin plants (B) and cage with field margin plants (C).	64

LIST OF TABLES

Table 3. 1: Mean monthly rainfall, relative humidity and temperature data observed during study period- May to December 2019 and March to November 2020	16
Table 3.2: Active ingredients and dose rates of botanical insecticides and synthetic insecticide used in the study.....	18
Table 3.3: Aphid abundance assessment scale	19
Table 3.4: Damage severity scoring scale.....	20
Table 3.5: Mean ($\pm$ SE) of aphid abundance, damage severity and percent incidence in margin vegetation during the 2019 and 2020 cropping cycles.....	24
Table 3.6: Abundance (Mean $\pm$ SE; n=4) of aphids in dolichos bean as affected by selected botanical insecticides and field margin vegetation (May-December 2019 cropping cycle).....	26
Table 3.7: Abundance (Mean $\pm$ SE; n=4) of aphids in dolichos bean as affected by selected botanical insecticides and field margin vegetation (March-November 2020 cropping cycle).....	27
Table 3.8: Severity (Mean $\pm$ SE; n=4) of damage caused by aphids on dolichos bean as affected by selected botanical insecticides and field margin vegetation (May-December 2019 cropping cycle).....	30
Table 3.9: Severity (Mean $\pm$ SE; n=4) of damage caused by aphids on dolichos bean as affected by selected botanical insecticides and field margin vegetation (March-November 2020 cropping cycle).....	31
Table 3.10: Incidence (Mean $\pm$ SE; n=4) of aphids in dolichos bean as affected by selected botanical insecticides and field margin vegetation (May-December 2019 cropping cycle).....	34
Table 3.11: Incidence (Mean $\pm$ SE; n=4) of aphids in dolichos bean as affected by selected botanical insecticides and field margin vegetation (March-November 2020 cropping cycle).....	35
Table 3.12: Number of parasitic wasps, tachinid flies and ladybird beetles as influenced by presence or absence of field margin vegetation and trapping area	37
Table 4.1: Mean ($\pm$ SE) of undamaged pods/plant, damaged pod/plant, seeds/pod, 100seedweight, grain yield and biomass in margin vegetation during 2019 and 2020 cropping cycle	55
Table 4.2: Mean ($\pm$ SE) of undamaged pods/plant, damaged pod/plant, seeds/pod, 100seedweight, grain yield, biomass and plant height as affected by selected	

botanical insecticides and field margin vegetation during May_ December 2019 cropping cycle.....	57
Table 4.3: Mean ($\pm$ SE) of undamaged pods/plant, damaged pod/plant, seeds/pod, 100seedweight, grain yield, biomass and plant height as affected by selected botanical insecticides and field margin vegetation during March_ November 2020 cropping cycle.....	58
Table 5.1: Population densities (Mean $\pm$ SE) of ladybird beetles on dolichos bean as influenced by botanical insecticides in the presence of field margin vegetation.....	68
Table 5.2: Population densities (Mean $\pm$ SE) of ladybird beetles on dolichos bean as influenced by botanical insecticides in the absence of field margin vegetation.....	71

LIST OF ABBREVIATIONS AND ACRONYMS

a.i	Active ingredient
ANOVA	Analysis of variance
BYMV	Bean Yellow Mosaic Virus
CPD	Change in population density
DAP	Days after planting
DAS	Days after spraying
FMV	Field margin vegetation
IPM	Integrated Pest Management
KALRO	Kenya Agricultural and Livestock Organization
MSD	Minimum significant difference
RCBD	Randomized Complete Block Design
RP	Reduction in population
SAS	Statistical Analysis Software

CHAPTER ONE

INTRODUCTION

1.1 Background information

Dolichos bean (*Lablab purpureus* L.) has been categorised as one of the potential climate-resilient crops with multiple uses. The crop can be used as food for human beings, fodder for livestock and soil fertility reclamation through biological nitrogen fixation and green manure (Grotelüschen *et al.*, 2014; Maass *et al.*, 2010; Mondal *et al.*, 2017; Nord *et al.*, 2020). Although dolichos appears to have originated in Africa, its largest agro-morphological diversity occurs in South Asia (Maass, 2016; Maass *et al.*, 2010). Currently, dolichos is grown throughout the tropical and subtropical regions of Asia, Africa and America (Maass *et al.*, 2010; Mondal *et al.*, 2017). In Africa, dolichos are widely grown in Kenya (Machakos, Kitui, Makueni, Tharaka-Nithi, Laikipia and Nakuru counties) and Tanzania (Arusha, Manyara, Kilimanjaro, Mbeya, Dodoma, Tanga and Morogoro districts) (Chawe, 2019; Grotelüschen *et al.*, 2014). According to Kamotho (2015), dolichos production in Kenya is carried out by smallholder farmers on a land area less than 0.4ha. Grain yields of 0.8-0.9 tons per hectare have been reported on farmers' fields which is less than the anticipated production of 2.7 to 3.0 tons per hectare (Ngure *et al.*, 2021). Due to low production, dolichos is considered a neglected legume and has been categorised as an orphan crop (Kilonzi *et al.*, 2017; Raghu *et al.*, 2018).

Dolichos is well adapted to a wide range of environmental conditions growing in semi-arid, subtropical and humid regions (Forsythe, 2019; Grotelüschen *et al.*, 2014; Raghu *et al.*, 2018). However, insect pests and diseases are considered to be a major constraint to its production (Boit *et al.*, 2018). Among the insect pests, pod borers (*Helicoverpa armigera* and *Maruca vitrata*) and the black bean aphid (*Aphis fabae*) are considered major pests (Boit *et al.*, 2018; Cork *et al.*, 2009). Black bean aphids form colonies around the stem, leaves and meristems causing yellowing of leaves, desiccation, stunting in older plants and sometimes death of affected plants (Mwangi *et al.*, 2008). Yield losses of 30% to 90% due to aphids on related legumes such as common beans (*Phaseolus vulgaris* L.) have been reported in East Africa (Munyasa, 2013). Boit *et al.* (2018) reported that an 80% yield reduction in dolichos can be attributed to sap-sucking insect pests. Besides causing direct damage to the host, bean aphids transmit diseases such as Bean Yellow Mosaic Virus (BYMV) which is considered the most damaging feature of infestation (Singh & Singh, 2017). Bean aphids also secrete honeydew which enhances the growth of sooty moulds reducing the photosynthetic ability of the plant.

The use of broad-spectrum synthetic insecticides has been recommended to control aphids as an emergency response to slow the spread and minimize the damage caused by aphids (Stevenson *et al.*, 2017). Although synthetic insecticides play an important role in aphid management, their negative effects on non-target organisms, the environment and the health of farmers and consumers continue to be problematic (Mkenda *et al.*, 2015, 2019). These side effects have led to increasing interest in the adoption of safe alternatives in controlling insect pests of beans such as agronomic practices, biological and botanical insecticides control. Integrated pest management (IPM) programs emphasize the combination of different control methods to maintain pest populations below economic injury levels and minimise non-target effects (Amoabeng *et al.*, 2020).

Biological control is an important component of IPM which focuses on the use of natural enemies such as predators and parasitoids to manage insect pests. Conservation of natural enemies of aphids through the provision of a suitable environment for these organisms can serve to inhibit pest build-up in fields (Nyaanga, 2008). Natural enemies can be conserved through the creation of non-crop vegetation and the use of selective insecticides (Roubos *et al.*, 2014). A valuable non-crop habitat type is areas of flowering plants in and around crop fields, and this has been employed in various systems to support beneficial insects and increase their abundance for biological control (Paredes *et al.*, 2013; Ramsden *et al.*, 2014).

The use of selective botanical insecticides is an important approach toward the conservation of biological control agents as the active ingredients of these insecticides are non-persistent presenting a lower risk to farmers and non-target organisms (Sola *et al.*, 2014). In recent studies, botanical extracts have shown low mortality in beneficial insects compared to synthetic products (Mkenda *et al.*, 2015). However, commercial botanical insecticides have not been tested for efficacy against major dolichos bean insect pests or effects on natural enemies of aphids. Therefore, this study evaluated the integration of botanical insecticides and field margin vegetation in the conservation of natural enemies in pest control on orphan crop legumes.

1.2 Statement of the problem

Bean aphid is a major insect pest in dolichos bean production that attack crops by sucking sap from various parts of the plant and is a vector of crop viruses. Yield losses ranging from 30% to 90% due to aphid infestation have been reported in East Africa with losses in Kenya being about 37%. Populations of natural enemies are subjected to continuous reduction, especially in modern agricultural systems characterised by the complete removal of plants and

overreliance on synthetic insecticides. Sustainable pest management options have not been widely studied on dolichos bean and how field margin vegetation mitigates negative impacts of synthetic insecticides use or facilitates benefits towards conservation biological control. Dolichos bean is an underutilised legume that could be used as a model crop to test the integration of such strategies on orphan crops which often lack good agricultural practices to manage insect pests. The indiscriminate continuous application of synthetic insecticides with no provision of floral resources such as pollen and nectar has resulted in the disappearance of natural enemies after each crop cycle. Pyrethrum and neem products are well-established commercial pesticides based on known active ingredients (natural pyrethrins and tetranortriterpenoids). However, the adoption of these botanical insecticides is limited due to variable efficacy against target pests. The effect of declining natural enemy populations have further resulted in severe damage by insect pest which was of little economic importance. Moreover, the occurrence, population density and diversity of most natural enemies in the smallholder farming systems are poorly understood, hence there is a need to develop and promote environmentally benign approaches that support and augment natural pest regulation.

1.3 Objectives

1.3.1 Broad objective

To contribute towards food and nutrition security through the integration of botanical insecticides and field margin vegetation for management of aphids and conservation of natural enemies in legumes.

1.3.2 Specific objectives

- i. To determine the effect of botanical insecticides and field margin vegetation on bean aphids and natural enemy populations in dolichos bean.
- ii. To determine the effect of botanical insecticides and field margin vegetation on the growth and yield of dolichos bean.
- iii. To determine the effect of botanical insecticides and field margin vegetation on recovery of ladybird beetles in dolichos bean.

1.4 Hypotheses

- i. Botanical insecticides and field margin vegetation have no significant effect on bean aphids and natural enemy populations in dolichos bean.

- ii. Botanical insecticides and field margin vegetation have no significant effect on the growth and yield of dolichos bean.
- iii. Botanical insecticides and field margin vegetation have no significant effect on the recovery of ladybird beetles in dolichos bean.

1.5 Justification of the study

Dolichos bean is greatly utilised as human food and animal feed though it remains neglected and underutilised. Due to these established uses of dolichos, there is a need to provide priority to promoting this multi-purpose legume in both commercial and smallholder farming systems to bridge the gap of zero hunger. Severe insect pest damage is one of the major constraints in lowering yield potentials. Synthetic insecticides offer a quick solution to reducing crop damage and yield losses. However, the side effects of synthetic insecticides, such as high mortality in non-target beneficial insects and increased risks to human health and the environment, have led to the development of environmentally friendly pest management strategies. Integration of locally available non-crop habitats such as flowering weed species provides the environment with diverse food resources (nectar and pollen) required to support arthropod predators and parasitoids. The use of botanical insecticides alongside natural enemy conservation potentially offers an integrated and effective alternative to synthetic insecticides for pest control. Conservation biological control and botanical insecticides have been used in managing field pests in isolation hence there is need to combine strategies for better long term pest suppression. Bean aphids have numerous natural enemies that could be conserved to minimize the use of broad-spectrum insecticides. Therefore, there is a need to evaluate the field performance of botanical insecticides together with field margin vegetation on insect pests and to understand their impact on natural enemies as well as yield on orphan crop legumes.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and botany of Dolichos bean

Dolichos bean (*Lablab purpureus* L.) is one of the most diverse domesticated legumes regarding agro-morphological characteristics and uses (Maass *et al.*, 2010). Wild dolichos plants occur only in eastern and southern Africa and are considered the centre of origin, with Ethiopia as one of the potential centers of origin (Maass, 2016). Dolichos is so widely distributed in the Indian subcontinent and it constitutes a centre of secondary diversity (Raghu *et al.*, 2018). Dolichos belongs to the family Fabaceae and it is known by several common names such as hyacinth bean, Kara-Kara, Komak and Ngwara among others depending on the country (Maass *et al.*, 2010), in Kenya, it is referred to as *Njahe* among Kikuyu community (Grotelüschen *et al.*, 2014).

The species is highly diverse and the subspecies are recognized, mainly based on different characteristics of growth habits, flowers, pods and seeds (Maass *et al.*, 2005; Pengelly & Maass, 2001). It is a semi-erect perennial herbaceous, erect cultivar the stem can grow up to 0.9meters and the climbing cultivars can grow up to 7metres (Guretzki & Papenbrock, 2013; Mondal *et al.*, 2017). Dolichos has an extensive root system with a vigorous taproot with the ability to extract soil water from a depth of 2m making it drought-tolerant (Grotelüschen *et al.*, 2014; Kilonzi *et al.*, 2017). It has long-stemmed trifoliate leaves; leaflets can be either triangular or oval in shape 7-15cm long depending on the cultivar. The inflorescence is made up of racemes flowers with variations in petal colours from purple, blue and white flowers (Maass *et al.*, 2010). Pods are 4-10cm long, smooth, flat, pointed and contain 2-5 seeds depending on the cultivar and environmental conditions. Seeds can be white, cream, pale brown, dark brown, black, or mottled (Mondal *et al.*, 2017; Reddy *et al.*, 2018).

2.2 Climatic requirements for Dolichos bean production

Dolichos is adapted to a wide range of environmental conditions which to some extent is attributed to its great genetic diversity (Grotelüschen *et al.*, 2014; Maass *et al.*, 2010). It is grown in the tropics, subtropics and humid climates (Maass *et al.*, 2005, 2010). The altitude ranges 400-2500 meters above sea level (masl) but lower altitudes are most ideal, usually not exceeding 2000 masl (Guretzki & Papenbrock, 2014). It does well in areas where the annual temperature ranges between 18-30 °C, with a minimum temperature of 3°C (Ewansiha *et al.*, 2007). High temperatures have been shown to slow vegetative development while frosts can

damage leaves (Schultze-Kraft *et al.*, 2018). Due to its wide adaptation, dolichos can be grown in areas with 200- 2500mm of annual precipitation (Morris, 2009). However, for the successful establishment of a deep root system in drier regions, irrigation during the first 1-3 months after planting is important (Nahashon *et al.*, 2016). Once established, dolichos are hardy, drought-tolerant and can grow to produce flowers and pods for many months and are even able to stay green during the dry cycle (Grotelüschen *et al.*, 2014). Dolichos can be grown on a wide range of soil textures provided they are well-drained (Cheruiyot *et al.*, 2011). It does not tolerate water logging and nodulates easily with dolichos or cowpea (*Vigna unguiculata*)- type *Rhizobium spp.* which are common in tropical and subtropical soils (Guretzki & Papenbrock, 2014). Dolichos has been mentioned to exhibit tolerance to low soil fertility with pH regimes ranging from 5.9 to 7.8 (Robotham & Chapman, 2017).

2.3 Production and utilization of Dolichos bean

Production of dolichos in Africa is spread out in small regions of East Africa (Uganda, Tanzania and Kenya) and West Africa (Cameroon and Nigeria) (Forsythe, 2019). 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% less 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, 000ha 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 have high nutritive qualities yet their global cultivation and utilization are extremely low hence considered underutilised (Maass *et al.*, 2010; Varshney *et al.*, 2009). Dolichos is either cultivated as a pure 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 naturally rich in carbohydrates, proteins, fat and fibre as well as minerals which include Calcium, Phosphorous and Iron (Kilonzi *et al.*, 2017). The protein content of pods and seeds has been profiled to range from 10-19% and 15-25%, respectively (Naeem *et al.*, 2009). Protein isolates from dolichos have the potential as a food additive for improving cake quality such as volume development, texture, colour and staling rate (Subagio & Morita, 2008). The

fodder dolichos bushy types can either be conserved into hay or fed green to dairy cattle (Guretzki & Papenbrock, 2014; Maass *et al.*, 2010). Dolichos as green manure has been demonstrated to improve soil available nitrogen (Cheruiyot *et al.*, 2011; Northup & Rao, 2015).

2.4 Constraints to production and yield of Dolichos bean

Dolichos bean is a neglected and underutilised legume crop in Africa and other parts of the world (Maass *et al.*, 2010; Nahashon *et al.*, 2016). The production is largely done by smallholder farmers in the marginal areas and faces numerous challenges (Grotelüschen *et al.*, 2014; Pauline, 2020). These challenges include low soil fertility, insect pest and disease infestation, poor agronomic practices and erratic rainfall distribution (Forsythe, 2019). These constraints cause a severe reduction in growth which is reflected in yield (Faria *et al.*, 2016).

Diseases are some of the major biotic factors which result in poor plant vigour, low yield and poor quality seed (Buruchara *et al.*, 2010). Dolichos can be affected by fungal diseases such as anthracnose (*Colletotrichum lindemuthianum*), Rust (*Uromyces appendiculatus*), powdery mildew (*Erysiphe polygoni*), Angular leaf spot (*Phaeoisariopsis greseola*). Similarly, they may be affected by bacterial diseases such as Halo blight (*Pseudomonas phaseoliola*) and common blight (*Xanthomonas campenstris* Pv *phaseoli*) (Kilonzi *et al.*, 2017). The two viral diseases, Bean Common Mosaic Virus and Bean Yellow Mosaic Virus are also important and are transmitted by aphids (Singh & Singh, 2017).

Several studies have reported that insect pests are one of the biotic factors that significantly reduce the yield of dolichos accounting for up to 50% of the total losses (Boit *et al.*, 2018; Forsythe, 2019; Mondal *et al.*, 2017; Pervez & Chandra, 2018). Pests' severity and incidence of attack vary depending on the location, varieties used, prevailing environmental conditions and agronomic practices used (Mwanauta *et al.*, 2015). Insect pests of economic importance that attack dolichos can be grouped according to the plant growth stage: seedlings, bean fly (*Ophiomyia phaseoli* and *O. centrosematis*) and foliage beetles (*Ootheca beningseni* and *Acanthomia horrid*), flowering, bean flower thrips (*Megalurothrips sjostedti*), podding, legume pod borers (*Helicoverpa armigera* and *Maruca vitrata*) and storage, bruchids (*Acanthosalides obtectus*) (Abate & Ampofo, 2003; Mkindi *et al.*, 2017). However, the black bean aphid (*Aphis fabae*) attacked dolichos at all growth stages (Valenzuela & Hoffmann, 2015).

Aphids (Hemiptera: Aphididae) are among the most important insect pest in dolichos and other legume crops including soybean (*Glycine max*), pigeon pea (*Cajanus cajan*) and common bean (*Phaseolus vulgaris*) (Mwanauta *et al.*, 2015; Pervez & Chandra, 2018; Reddy

et al., 2017). Aphids reproduce very fast within a short period to achieve high population density resulting in economic yield loss (Mondal *et al.*, 2017). Aphids are diverse and species vary depending on the host plant (Bachmann *et al.*, 2014). The black bean aphid (*Aphis fabae*) is the most dominant species that attack dolichos (Boit *et al.*, 2018; Mondal *et al.*, 2017; Singh & Singh, 2017).

2.5 Distribution and biology of bean aphid (*Aphis fabae* S.)

Aphids are polyphagous species widely distributed in tropical, subtropical and temperate regions of the world infesting legumes and cereals crops as well as vegetables (Valenzuela & Hoffmann, 2015). The black bean aphid (*Aphis fabae* S.) is the principal aphid pest that causes direct and indirect damage to beans in Africa (Mwanauta *et al.*, 2015; Mwangi *et al.*, 2008). It is one of the most widespread aphid species and displays a vast range of host plants (Singh & Singh, 2017).

The black bean aphid belongs to the phylum arthropod, class Insecta, order Hemiptera, family Aphididae, genus *Aphis* and species *A. fabae* (Emmanuelle *et al.*, 2010; Gutierrez *et al.*, 2006). The black bean aphid is a small soft-bodied insect that has specialized piercing and sucking mouthparts that are used to suck plant sap (Singh & Singh, 2017). The aphids are usually seen in large colonies as the population builds up with adults approximately 2mm long with a small head and bulbous abdomen (Abate & Ampofo, 2003; Mondal *et al.*, 2017). The antennae are less than two-thirds their body length and both legs are pale yellow with black tips (Dixon, 2019). Near the rear segments of the abdomen is a pair of slender elongated tube, cornicle which produces a defensive waxy secretion (Kindlmann & Dixon, 2010).

The aphid life cycle is complex and typically consists of several asexual generations alternating with a single sexual generation (Buruchara *et al.*, 2010). The aphid life cycle takes 11-13 days and adult longevity varies from 6 to 15 days (Dixon, 2019). In the warm tropics of Africa, reproduction is only parthenogenetic (Abate & Ampofo, 2003). During the phase of asexual reproduction, aphids produce several different phenotypes, among which are winged and wingless forms (Petermann *et al.*, 2010; Weisser, 2019). Wingless forms are produced when food is abundant and climatic conditions are optimal. However, when food is inadequate or the colony becomes overcrowded, winged forms develop (Mondal *et al.*, 2017). The winged forms migrate to colonize new fields and may invade bean fields soon after seedling emergence (Singh & Singh, 2017).

2.6 Nature and extent of damage caused by bean aphid

Bean aphids cause economic damage through direct feeding and indirect damage due to virus transmission (Valenzuela & Hoffmann, 2015). Bean aphids form around the stem, leaves and meristems throughout the growth crop stages (Figure 2.1). During sap-sucking, aphids divert plant nutrients for their benefit reducing plant growth (Munyasa, 2013). In most cases, plants respond to aphid feeding by leaf rolling, yellowing and necrosis (Singh & Singh, 2017). When aphids are in large colonies honeydew excretion increases which cover the leaf cuticle and is colonised by sooty moulds reducing the photosynthetic ability of the plants (Lundgren, 2009).

Besides causing direct damage to the host by sucking the sap from various plant parts, they also indirectly transmit common mosaic viruses which result in early plant death (Munyasa, 2013). The indirect damage that aphids cause through virus transmission often far exceeds their direct impact on crops (Valenzuela & Hoffmann, 2015). The aphid mouth part consists of a slender stylet which is very suitable for tapping the nutrient-rich sieve element sap (Singh & Singh, 2017). Mondal *et al.* (2017) reported that aphid stylet follows a predominantly intercellular route to minimize the cell damage that reduces the plant defense response on their way to reach the sieve element cell in the vascular tissue.

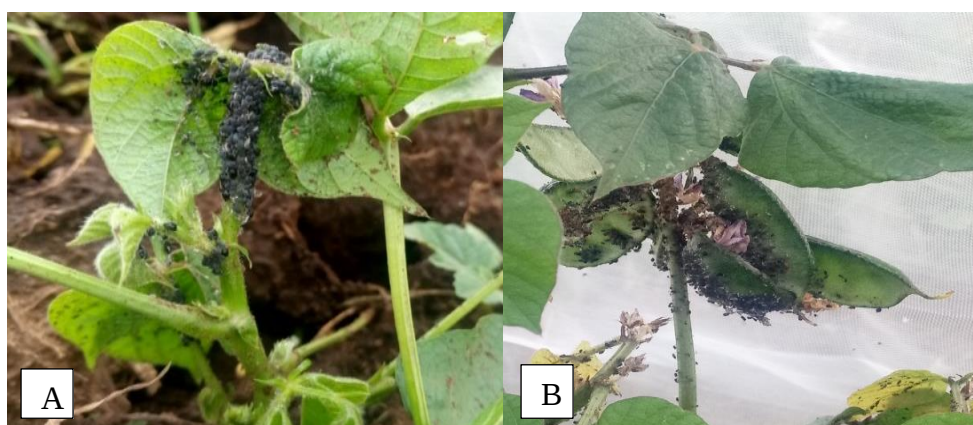


Figure 2. 1: Black bean aphids (*Aphis fabae* S.) on dolichos at: (A) early vegetative stage and (A) podding stage

2.7 Control methods of bean aphids

Bean aphids are phytophagous insect pests that are difficult to control through the use of one particular method (Mwanauta *et al.*, 2015). Aphid populations usually build up during dry weather thus, infestations are more severe during the dry cycle (Wosula *et al.*, 2017). However, insect pest control is often overlooked on orphan legumes including dolichos and

any control measure is dependent on chemical pesticides which are detrimental to the health of farmers, consumers, non-target organisms and the environment (Stevenson *et al.*, 2017). Bean aphids have a high reproductive rate posing an immense challenge in control using a single strategy (Mwanauta *et al.*, 2015). Hence there is a need to develop other control strategies with aim of integrating two or more methods that are safe and environmentally friendly. Farmers have adopted different strategies to manage insect pests of legumes including cultural practices, chemical insecticides, pesticidal plants and biological methods (Karani, 2017).

2.7.1 Cultural control

Cultural practices are the oldest methods that have been used to manage pest populations (Kebede *et al.*, 2019). However, with the development of synthetic pesticides, cultural methods were rapidly abandoned and research on them was largely discontinued (Abate & Ampofo, 2003). The use of cultural methods largely depends on detailed knowledge of the bio-ecology of the crop pests, natural controls and environmental relationships (Wosula *et al.*, 2017). Cultural controls adopt practices that make the environment less attractive to pests and less favorable for their survival, dispersal, growth and reproduction and that promote pest's natural controls. The main aim of cultural controls is to achieve a reduction in pest numbers, either below the economic injury level, or sufficiently allow biological controls to take effect (Shehu *et al.*, 2001).

The majority of smallholder farmers often intercrop beans with other crops including cereals such as finger millet (*Eleusine indica*), sorghum (*Sorghum bicolor*) and maize (*Zea mays*) (Grotelüschen *et al.*, 2014). These practices cannot be effective in isolation but a combination of several practices is necessary for effective control (Kebede *et al.*, 2019). In a related study, (Abate & Ampofo, 2003) observed that bean fly (*Ophiomyia spp*) adults only emerged in the presence of beans or other hosts such as cowpea seedlings but not in the presence of non-host such as maize in the field. Trials in the laboratory showed the larvae survived on bean and cowpea roots but failed on soybean and maize roots. Cultural practices are considered safe and cheap, hence their integration into insect pest management programmes will reduce reliance on chemical insecticides (Mwanauta *et al.*, 2015).

2.7.2 Synthetic insecticides control

Over the last 60 years, farmers have achieved significant progress in food production by using insecticides (Sola *et al.*, 2014). Synthetic insecticides are considered to be fast-acting and can kill a wide range of insect pests but have several limitations attributed to the killing of

both beneficial and non-beneficial insects (Stevenson *et al.*, 2017). Despite their usefulness and effectiveness, synthetic insecticides have limited distribution in rural areas, often adulterated or applied at an inappropriate application rate (Karani *et al.*, 2017). Insecticides including cypermethrin, carbamates and pyrethroids have been reported with high efficacy in controlling insect pests (Maksymiv, 2015; Ndakidemi *et al.*, 2016). However, with the rapid move toward safe alternatives, selective synthetic insecticides have been developed such as indoxacarb and spinosad (Stoddard *et al.*, 2010). These chemicals are considered to be selective and are less harmful to beneficial insects such as natural enemies and pollinators as well as being effective in controlling insect pests. In a related study, Mwanauta *et al.* (2015) reported not only reduced leaf miner *Liriomyza huidobrensis* (Blanchard) populations but also suppressed its parasitoid when imidacloprid was applied as a foliar spray at a podding stage on common bean (*Phaseolus vulgaris*).

Synthetic pyrethroids have been widely used for the control of insect pests on field crops (Bibi *et al.*, 2014). However, detrimental effects to human health, natural enemies and pollinators have been reported to be severe (Mwanauta *et al.*, 2015). Food safety concerns have increased the demand for organically produced food, which has coincided with environmental conservation (Chaudhary *et al.*, 2017). Therefore, there is a need for safe alternatives that present a low health risk and conserve the agricultural ecosystem biodiversity such as botanical insecticides and natural pest regulation.

2.7.3 Botanical insecticides control

Different plant extracts have been shown to contain a wide spectrum of secondary metabolites such as phenolics, flavonoids, quinones, tannins, alkaloids, saponins, sterols and essential oils known to effectively inhibit insect pest growth and development (Stevenson *et al.*, 2017). The plant secondary metabolites represent a large array of chemical structures with a biological activity that has been exploited as the active ingredients for botanical insecticides (Pavela, 2016). The bioactivity of plant derivatives to arthropod pests continues to expand, yet only a handful of botanicals are currently used in agriculture (Sola *et al.*, 2014). Currently, botanical insecticides comprise 5–6% of the total global crop pest control in the market, with a value of between \$3 and \$4 billion (Baker *et al.*, 2020).

Pyrethrum and neem products are well-established commercial pesticides based on plant essential oils (Chaudhary *et al.*, 2017). However, varying efficacy and low toxicity against target pests, which is partly due to the rapid breakdown of bio-active compounds have been a challenge for their application (Sola *et al.*, 2014). Consumers and policy makers are

demanding reduced synthetic inputs in food production and practices that support agro-ecological intensification and pesticidal plant products (Pavela, 2016; Sola *et al.*, 2014). Mkenda *et al.* (2015) found that extracts made from four abundant weed species in northern Tanzania, *Tithonia diversifolia* (Hemsl.) A. Gray, *Tephrosia vogelii* Hook., *Vernonia amygdalina* Delile and *Lippia javanica* (Burm.f.) Spreng, offered effective control of key pest species on common beans (*Phaseolus vulgaris* L.) comparable to the synthetic pyrethroid. Insecticidal properties of various bioactive molecules have been shown, for instance, pyrethrin is known to have a rapid knockdown effect, hyperactivity and convulsion in most insects (Stevenson *et al.*, 2017). The application of botanical insecticides has increased among the farmers. However, there is a need for research to evaluate the field performance of these botanical insecticides on major insect pests and their natural enemies on orphan crop legumes such as dolichos.

2.7.4 Biological control

Several groups of natural enemies are known to provide biological control of aphids (Schmidt *et al.*, 2003). Numerous predators, parasitoids and entomopathogens exploit aphid colonies (Alignier *et al.*, 2014; Skirvin *et al.*, 2011). Among predators, adults and larvae of coccinellid beetles (Coleoptera: Coccinellidae), larvae of lacewings (Neuroptera: Chrysopidae and Hemerobiidae), predatory midges (Diptera: Cecidomyiidae) feed on aphids. (Abd El-Salam *et al.*, 2012; Ahmad *et al.*, 2017; Mkenda *et al.*, 2019). Ladybird beetles are the most abundant and most effective aphid predators (Agarwala *et al.*, 2017). The effectiveness of ladybird beetles is attributed to their high individual rate of prey consumption, their great abundance, activity and their closer synchronization with aphid populations. (Grez *et al.*, 2014; Lundgren, 2009; Pervez & Chandra, 2018). Aphids are parasitized by several species of Hymenoptera and a few Diptera species including hoverflies (Diptera: Syrphidae) (Fite *et al.*, 2021). These parasitoids can cause mortality in aphid colonies and they are often used in biological control programs (Boivin *et al.*, 2012). Aphid parasitoids show different levels of host specialization (Ramsden *et al.*, 2014). Hymenopterous parasitoids are believed to mature on one aphid and therefore would appear to be potentially more likely to regulate aphid abundance (Prado *et al.*, 2015). Entomopathogenic fungi, mostly the Deuteromycotina and Zygomycotina, are the most common aphid pathogens (Abd El-Salam *et al.*, 2012)

Several biological approaches can be used to control aphids (Nyaanga, 2008). The three main approaches include classical, augmentation and conservation biological control. Classical biological control consists of the introduction of an exotic natural enemy for the regulation of

a pest species, itself usually introduced accidentally outside of its natural distribution. Augmentation of biological control entails supplementing existing natural enemies through mass-rearing and releasing them to the field. Finally, conservation biological control comprises enhancing the natural enemies in-situ (Ahmad *et al.*, 2017; Sorribas *et al.*, 2016).

2.8 Conservation of natural enemies

Conservation of natural enemies aims at environmental modifications to preserve and protect natural enemies from adverse conditions (Paredes *et al.*, 2013). Natural enemies can be conserved through the use of selective insecticides, low doses and the creation of non-crop vegetation (Roubos *et al.*, 2014). Selective insecticides may improve the conservation of natural enemies and therefore contribute to the success of integrated pest management (Khan *et al.*, 2015). Botanical insecticides have been reported to cause minimal harm to predators and parasitoids due to their mode of action such as repellence, anti-feeding and oviposition deterrence rather than acute toxicity (Braumah *et al.*, 2014). A sustainable alternative to synthetic insecticides has been proposed: conservation biological management based on the establishment of non-crop vegetation and the use of selective insecticides (Amoabeng *et al.*, 2020). Natural enemies of pest species 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).

Integrated pest management combines various pest management strategies to maintain the pest population below the economic injury level (Roubos *et al.*, 2014). However, the combination of the various methods must not result in major lethal or sub-lethal effects on non-target organisms especially natural enemies that are the cornerstone of natural pest regulation (Gurr *et al.*, 2017). Although both conservation biological control and botanical insecticides have been used in managing field pests, combining the two strategies require careful assessment of their individual and combined effects on pests and natural enemies as well as the overall impact on crop yield. Many studies have reported the effect of non-crop vegetation and botanical insecticides in isolation on both pests and natural enemies with varying results (El-Wakeil, 2014). Integration of botanical insecticides and conservation biological control may result in better pest suppression as compared to each tactic in isolation. To address this gap, a study was designed to determine the effects of field margin vegetation and selected commercial botanical insecticides on aphids and their natural enemy populations in dolichos bean.

CHAPTER THREE

EFFECT OF BOTANICAL INSECTICIDES AND FIELD MARGIN VEGETATION ON BEAN APHIDS AND NATURAL ENEMY POPULATIONS IN DOLICHOS BEAN (*Lablab purpureus* L.)

Abstract

Insecticides are primarily applied to reduce damage caused by insect pests with no or little emphasis on potential side effects to natural enemies. A field study was conducted over two cropping cycles (2019 and 2020) to determine the efficacy and effectiveness of commercial botanical insecticides: Pyerin75EC[®] (1% natural pyrethrin + 1% neem oil + 25% garlic extracts), Nimbecidine[®] (0.03% Azadirachtin + 90.57% neem oil) and Pyeneem 20EC[®] (1% natural pyrethrin + 1% neem oil) tested alongside synthetic insecticide Duduthrin 1.75EC[®] (1.75% *lambda-cyhalothrin*) and untreated as positive and negative controls, respectively on dolichos bean aphids and their natural enemies. Treatments were laid out in a randomized complete block design (RCBD) in which field plots were either surrounded by field margin vegetation (FMV) or with no FMV. The botanical insecticides and Duduthrin were applied at the second and sixth trifoliolate growth stages. Results showed that botanical insecticides and synthetic insecticides reduced aphid abundance by 88 and 80%, respectively compared to the untreated control. In plots surrounded with FMV and treated with botanical insecticides higher number of natural enemies (33.55%) were collected as compared to no FMV (24.88%). The presence of field margin vegetation increased the number of parasitic wasps and tachinid flies by 16% and 20%, respectively. The study demonstrated that commercial botanical insecticides together with field margin vegetation are eco-friendly and viable alternatives to synthetic insecticides for insect pest management in legume crops.

3.1 Introduction

The black bean aphid, (*A.fabae*) is an important pest of legume crops including dolichos (Buruchara *et al.*, 2010). The bean aphid feeds by sucking sap from leaves, stems and buds (Singh & Singh, 2017). Their piercing-sucking feeding habit extracts plant sap, causing yellowing and curling of leaves, retarded growth and deformed pods (Dively *et al.*, 2020; Nyaanga, 2008). Severe bean aphid infestation leads to delayed plant growth and development with the subsequent effect of low crop yield (Mwanauta *et al.*, 2015; Wosula *et al.*, 2017)

Chemical insecticides make the most rapid and practical way to manage bean aphids because of their high-rated efficacy (Pezzini & Koch, 2015; Sisay *et al.*, 2019). The dependence

on chemical insecticides has resulted in several ecological and environmental constraints for humans, animals and beneficial insects (Depalo *et al.*, 2017; Ndakidemi *et al.*, 2016). Insect pests and their natural enemies have been shown to possess physiological similarities and the insecticides applied to reduce the population in both groups (Gholamzadeh-Chitgar & Pourmoradi, 2017). Therefore, knowledge of the effect of insecticides applied for the control of bean aphids on their natural enemies is essential to find relatively safe alternatives that conserve the natural biological agents.

Natural enemies can be conserved through the creation of non-crop vegetation and the use of selective insecticides (Roubos *et al.*, 2014). Provision of supplementary non-crop habitat by using flowering plants in and around crop fields has been proposed as a means of supporting and increasing the abundance of beneficial insects useful for biological control (Trisnawati & Azis, 2017). The use of selective insecticides together with the conservation of natural enemies could help reduce reliance on synthetic insecticides for insect pest control. Due to a growing awareness of the harmful side effects of synthetic insecticides, alternate methods of pest control with extremely safe insecticides are being promoted. There is an increasing interest in the use of botanical insecticides in the management of insect pests which are considered safe and selective to a wider group of natural enemies (Fernandes *et al.*, 2010; Monsreal-Ceballos *et al.*, 2018). Benefits of using botanical pesticides such as reduced environmental degradation, increased safety for farm workers, increased food safety, reduction in pesticide resistance and improved profitability of production have been reported (Mkindi *et al.*, 2017; Mwanauta *et al.*, 2015). Therefore, this study evaluated the effect of botanical insecticides and field margin vegetation on the black bean aphid and its natural enemy population compared to synthetic insecticide, Duduthrin in dolichos beans.

3.2 Materials and methods

3.2.1 Experimental site

The studies were carried out at the Agronomy Research and Teaching field, Egerton University, Njoro (0° 20' S and 35° 56' E), Nakuru County, Kenya. The site is located at an altitude of 2250m above sea level. The site is considered an agriculturally high potential agro-ecological zone in lower highland 3 (LH3) within the Kenya Highlands (Jaetzold *et al.*, 2012). The area receives annual precipitation of about 1200 mm with a mean annual temperature of 17°C -22°C. The field trials were carried out from May to December 2019 and March to November 2020 cropping cycles and weather data during this period are shown in Table 3.1. The weather conditions were different in both cropping cycles. The annual precipitation

received was higher in 2019 (1105.6 mm) than in 2020 (700.7 mm). In 2019, the average maximum temperature was lower (22.9°C) and had higher relative humidity (70 %) than in 2020, which had a higher maximum temperature (23.1°C) and lower relative humidity (68 %) (Table 3.1). The soils are well-drained dark reddish clays, classified as Mollic Andosols.

Table 3.1: Mean monthly rainfall, relative humidity and temperature data observed during study period - May to December 2019 and March to November 2020

Month	2019 cycle				2020 cycle			
	Temp. (°C)		Rainfall	RH	Temp. (°C)		Rainfall	RH
	Max.	Min.	(mm)	(%)	Max.	Min.	(mm)	(%)
May	24.7	15.5	60.4	60	23.7	14.4	91.6	65
June	22.4	14.6	232.4	79	22.7	13.2	93.5	74
July	22.2	13.5	146.7	70	21.4	16.3	113.9	71
Augst.	22.5	13.1	76.4	69	21.3	18.2	119.2	76
Sept.	24.7	13.3	89.7	65	22.6	15.1	96.4	73
Oct.	22.9	14.1	161.6	70	24.7	17.4	85.2	64
Nov.	22.8	14.4	114.8	70	23.9	15.1	49.7	61
Dec.	21.1	14.3	223.6	75	24.3	16.8	51.2	63

RH = Relative humidity (%), Temp = Temperature, °C= degree celcius Max.=Maximum, Min.=Minimum; Source: Egerton University Engineering Meteorological Station, 2020

3.2.2 Experimental procedure

Field trials were carried out during May to December 2019 and March to November 2020 cropping cycles. The field was disc ploughed and harrowed before planting and plots measuring 10m × 10m and 10m apart were demarcated. The plot dimensions used were smaller than a typical field but were considered appropriate as related studies had been conducted using similar plot sizes (Hatt *et al.*, 2017). The first treatment level was for experimental blocks to be planted in the presence of field margin vegetation or for margin vegetation to be absent. Thus, two weeks before the bean crop was planted, field margin vegetation was sown with plant species to give the field margin plants time to establish. The plot margins were created with four common flowering weed species black jack (*Bidens Pilosa* L.), Mexican marigold (*Tagetes minuta* L.), goat weed (*Ageratum conyzoides* L.) and gallant soldier (*Galinsoga parviflora* Cav.). These species were chosen because they are annuals and occur in abundance

around the farms in the region. The selection was also guided by previous studies which indicated that these species affected the arthropod population (Amoabeng *et al.*, 2020; Quispe *et al.*, 2017; Souza *et al.*, 2019; Zhang *et al.*, 2021). The seeds were harvested on the experimental field before ploughing. The seeds of each species were mixed in equal proportions (by weight) and sown around each plot which had plant margin treatments. The margin species were planted 0.5m from the outer row of dolichos and 0.5m in width. To ensure uniform emergence of the plant species the planting area was prepared to a fine tilth. The experimental plots which had no margin plants were left bare by clearing any vegetation throughout the growing cycle. After field margin vegetation establishment (2 weeks after planting), dolichos bean variety DL-1002 was planted at a spacing of 60 cm by 30 cm, two seeds per hill, with an equivalent seed rate of 60 kg ha⁻¹. At planting, NPK (23:23:0) fertilizer was applied at the rate of 60 Kg N ha⁻¹ and 60 Kg P₂O₅ ha⁻¹.

The second treatment level involved the application of 3 commercial botanical insecticides: Pyerin 75EC[®], Pyeneem 20EC[®] (Twiga Chemical Industries Ltd, Nairobi, Kenya) and Nimbecidine[®] (T. Stanes and Co. Ltd, Coimbatore, India) and as well as a synthetic insecticide Duduthrin 1.75EC[®] (Twiga Chemical Industries Ltd, Nairobi, Kenya) as a positive control, and untreated as a negative control. Active ingredients and applied doses are described in Table 3.2. The experiment was carried out in 5 × 2 factorial arranged in a Randomized Complete Block Design (RCBD) with four replications. The insecticides were applied twice, with the spraying done at 42 days after planting (DAP) when the crop entered the second trifoliate and the second spraying was done at 70 DAP during the sixth trifoliate. These two growth stages were selected since aphids inflict severe damage at the vegetative growth stage, attacking auxiliary buds and growing points.

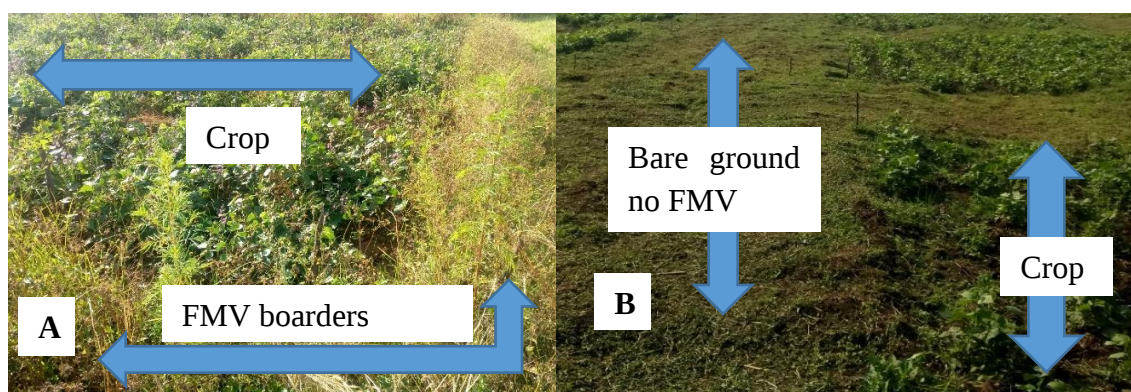


Figure 3. 1: Plots with field margin vegetation (FMV) (A) and without field margin vegetation (B) at Egerton University, Field 8

Table 3. 2: Active ingredients and dose rates of botanical insecticides and synthetic insecticide used in the study

Trade Name	Active ingredients (a.i., %)	a.i. dose (L/Ha)
Pyeneem	Natural pyrethrins 1% w/v	0.025
	Neem oil 1% w/v	0.025
	Inert ingredients 98% w/v	2.450
Pyerin	Natural pyrethrins 1% w/v	0.025
	Neem oil 1% w/v	0.025
	Garlic extracts 25% w/v	0.625
	Inert ingredients 73% w/v	1.825
Nimbecidine	Azadirachtin 0.03% w/v	0.0009
	Neem oil 90.57%	2.7171
	Inert ingredients 9.4% w/v	0.282
Dududthrin (+ve control)	Lambda cyhalothrin 1.75 % w/v	0.035
	Inert ingredients 98.25% w/v	0.197

*w/v = refers to weight by volume; a.i= active ingredient.

3.2.3 Data collection

Data on aphid abundance, severity and incidence were collected one day before spraying and 7, 14 and 21 days post spraying in each treatment and control. Aphid abundance was determined by visually observing and scoring aphid abundance levels. The data were collected from ten randomly selected plants from the inner five rows falling within the sampling area in each treatment. A categorical scale was used to assess aphid abundance, 1 = no aphids; 2 = A few scattered aphids; 3 = A few small colonies; 4 = Several small colonies; 5 = Large isolated colonies; and 6 = Large continuous colonies (Aken *et al.*, 2013; Mkenda *et al.*, 2015) (Table 3.3).

Table 3.3: 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	

Sources: Aken *et al.* (2013) and Mkenda *et al.* (2015)

Damage severity was determined by visually observing and scoring the level of aphid damage severity in each experimental unit from ten randomly selected plants in the inner five rows. The damage severity was assessed by scoring the extent of damage 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 3.4).

The aphid incidence was determined as a proportion of aphid damaged/infested plants to clean plants. This was done by visually examining and counting aphid-damaged or aphid-infested dolichos plants in each treatment. Incidences were obtained by randomly sampling 30 plants from the inner five rows in each treatment and were expressed as percentage incidence.

Table 3. 4: 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%	

Table 3.4 Continued...

Scale	Description of damage	Visual expression
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%	

Sources: Mkenda *et al.* (2015)

Sampling for Natural enemies

Assessments of ground-dwelling and flying arthropod groups were carried out one day before and 7 days after the spraying of the insecticides. Sampling was done by the deployment of yellow pan traps, yellow sticky traps and sweep nets to collect aphid natural enemies available at the crop center (5m from the edge) and field margin vegetation (Figure 3.2). The choice of the three different sampling methods was to capture the spatial distribution of natural enemies. Yellow pan traps were used to collect ground-dwelling natural enemies. The pan traps consisted of 0.2 m diameter and 0.5 m high plastic plates filled with water at a three-quarter level and droplets of soapy detergent were added to break the surface tension and a spoonful of table salt was added as a preservative. The pan traps were placed at ground level and were collected after 48 hours. The population of flying arthropod groups was determined by hanging yellow sticky cards (dimensions, 0.1 m by 0.25 m) 0.3 m above the crop. The sticky traps were left in the field for 48 hours. After 48 hours, the sticky traps were collected and placed in non-sticky laminating pouches (0.216 m x 0.303 m). The arthropod species in the sticky traps were removed using analytical hexane (85% AR) as described by Melanie *et al.* (2015). Sweep nets (dimensions 0.38 m diameter, 0.3 m deep and 0.9 m long metallic handle) were used to collect flying natural enemies. A total of tens sweeps were done in the field margin vegetation and the middle of the dolichos crop (5m from the margin) parallel to the margin sweep. The sweep net samples were first anesthetized by transferring to *Kilner* jars containing cotton wool soaked

with formalin. All arthropods captured in each sampling technique were transferred into 50 ml falcon tubes containing 75% ethanol. Arthropod samples were sorted and natural enemies of aphids were identified to the family level.

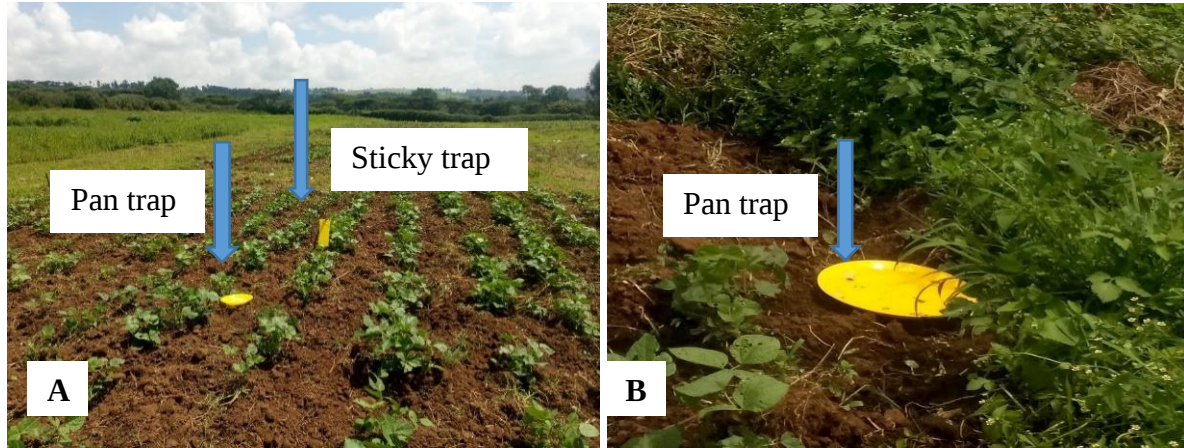


Figure 3.2: Yellow pan traps and yellow sticky traps used for natural enemies sampling at: (A) crop centre and (B) field margin vegetation.

3.2.4 Data analyses

Data on percent incidence and natural enemies counts were subjected to arcsine and square root ($\sqrt{x + 1}$) transformation, respectively to correct for heterogeneity of treatment variances. Effects of cropping cycles, botanical insecticides, field margin vegetation and their interactions subjected to Analysis of Variance (ANOVA) using Statistical Analysis System version 9.2 (SAS Institute, 2011) for aphids' abundance, damage severity, percent incidence and natural enemy abundance. The following statistical model was used for the analysis of variance for aphid abundance, damage severity, percent incidence and bean aphid natural enemy abundance.

$$Y_{ijklm} = \mu + F_i + R_j + G_k + FG_{ik} + I_l + FI_{il} + GI_{kl} + \mathcal{E}_{ijklm} \dots \dots \dots (3.1)$$

Where Y_{ijklm} is the observation of experimental units, μ Population means, F_i effect of i^{th} field margin vegetation, R_j effect of j^{th} replicate, G_k effect of k^{th} growth stage, FG_{ik} effect of interaction between i^{th} field margin and k^{th} growth stage, I_l effect of l^{th} insecticide, FI_{il} effect of interaction between i^{th} field margin vegetation and l^{th} insecticide, GI_{kl} interaction effect between k^{th} growth stage and l^{th} insecticide and $\mathcal{E}_{ijklm}$ random error.

The means of treatment and interactions were separated using Tukey's Honestly Significant Difference (HSD) test at a significant level of 5% (Gomez & Gomez, 1984; Tukey, 1949).

$$W = q[\alpha, p, fe] \times \sqrt{\frac{MSE}{r}} \dots\dots\dots (3.2)$$

Where W is the critical difference; p is the number of treatment means; fe is the degree of freedom of error; α is the level of significance; MSE is the mean square error and r is the number of replicates.

3.3 Results

3.3.1 Effect of cropping cycle and field margin vegetation on aphid abundance, damage severity and percent incidence

The results indicated that cropping cycle, field margin vegetation and botanical insecticides affected aphid abundance, damage severity and percent incidence. The cropping cycle significantly influenced aphid abundance ($P < 0.0001$), damage severity ($P < 0.0001$) and percent incidence ($P < 0.0001$) (Appendix A). Field margin vegetation significantly affected aphid abundance ($P < 0.001$), damage severity ($P < 0.001$), and percent incidence ($P < 0.001$). Similarly, botanical insecticide application significantly influenced aphid abundance ($P < 0.001$), damage severity ($P < 0.001$), and percent incidence ($P < 0.001$). There was a significant interaction effect between the cropping cycle and field margin vegetation for aphid abundance ($P < 0.001$), damage severity ($P = 0.0006$), and percent incidence ($P < 0.001$) (Appendix A). During the two cropping cycles plots with field margin vegetation had lower aphid abundance, damage severity and percent incidence as compared to plots with no field margin vegetation. Both in the presence and absence of field margin vegetation, in the 2020 cropping cycle, high aphid abundance (2.31, 2.83), damage severity (2.13, 2.56) and percent incidence (48.81, 60.13) were observed, respectively as compared to 2019 cropping cycle (Table 3.5).

Table 3.5: Mean ($\pm$ SE) of aphid abundance, damage severity and percent incidence in margin vegetation during the 2019 and 2020 cropping cycles

	May-December 2019 Cycle			March-November 2020 Cycle		
FMV	Aphid abundance	Damage severity	Percent incidence	Aphid abundance	Damage severity	Percent incidence
Present	1.69 $\pm$ 0.03 ^b	1.65 $\pm$ 0.02 ^b	44.96 $\pm$ 0.81 ^b	2.31 $\pm$ 0.04 ^b	2.13 $\pm$ 0.52 ^b	48.81 $\pm$ 0.74 ^b
Absent	2.07 $\pm$ 0.03 ^a	2.01 $\pm$ 0.03 ^a	53.59 $\pm$ 0.78 ^a	2.83 $\pm$ 0.05 ^a	2.56 $\pm$ 0.34 ^a	60.13 $\pm$ 0.74 ^b
MSD_(0.05)	0.02	0.03	0.95	0.03	0.04	0.74

Means in a column followed by the same letters are not significantly different using Tukey's HSD test at $P < 0.05$; FMV= Field margin vegetation; MSD= Minimum significant difference. Aphid abundance index: 1 = no aphids; 2 = A few scattered aphids; 3 = A few small colonies; 4 = Several small colonies; 5 = Large isolated colonies; and 6 = Large continuous colonies. Damage severity: 1= no damage, 2=light damage < 25 %, 3= damage 26 % - 50 %, 4= damage 51 % - 75 % yellowing of lower leaves; 5= damage >75 % yellow and severely curled.

3.3.2 Effect of botanical insecticides on aphids' abundance

During the 2019 cropping cycle, aphid abundance was significantly affected by botanical insecticide application ($P < 0.001$). Similarly, field margin vegetation and time before or after insecticide application were significant ($P < 0.001$) for aphid abundance. Compared to treated plots, aphid abundance increased over time in untreated control plots with the highest aphid abundance (2.50) observed 77 days after planting. In the absence of field margin vegetation reduction in the aphid abundance after botanical insecticides application was evident compared to untreated. At 21 days after spraying for the first spray, aphid abundance differed significantly ($P = 0.0006$) for the treatments. During the same period among the botanical insecticides, the highest aphid abundance was observed in plots treated with Nimbecidine (2.00) and the lowest in Pyerin (1.85) compared to Duduthrin (1.77) and untreated (2.47). At 21 days after the second spray, the lowest aphid abundance was observed in plots treated with Pyeneem (1.35) and the highest in Nimbecidine (1.52) compared to Duduthrin (1.22) and untreated (2.25) (Table 3.6)

In the 2020 cropping cycle, botanical insecticide application significantly influenced aphid abundance ($P < 0.001$). Field margin vegetation and time before or after spraying were also significant ($P < 0.001$). At 7 days after first spraying, the highest aphid abundance was observed in plots treated with Nimbecidine (2.07) and lowest in Pyeneem (1.80) compared to Duduthrin (1.70) and untreated (2.80). For the second spraying, the lowest aphid abundance

was observed at 21 DAS with the lowest abundance in plots treated with Pyeneem (1.57) compared to Duduthrin (1.62) (Table 3.7). In the absence of field margin vegetation, a reduction in the aphid abundance after botanical insecticide application was evident compared to untreated. At 21 days after first spraying, the lowest aphid abundance was observed in plots treated with Pyerin (2.50) compared to Pyeneem (2.52) and Nimbecidine (2.60). For the second spray at 21 DAS, the lowest aphid abundance was observed in plots treated with Pyeneem (1.92) followed by Pyerin (1.95) compared to Duduthrin (2.12) and untreated control (3.77) (Table 3.7).

Table 3.6: Abundance (Mean±SE; n=4) of aphids in dolichos bean as affected by selected botanical insecticides and field margin vegetation (May-December 2019 cropping cycle).

With field margin vegetation								
Botanicals	First Spraying				Second Spraying			
	Pre-spray 1	7DAS	14DAS	21DAS	Pre-spray 2	7DAS	14DAS	21DAS
Untreated control	2.02±0.11 ^a	2.00±0.11 ^a	2.10±0.00 ^a	1.90±0.08 ^a	2.20±0.04 ^a	2.50±0.07 ^a	2.45±0.06 ^a	2.25±0.06 ^a
Duduthrin	1.95±0.06 ^a	1.35±0.03 ^c	1.22±0.03 ^c	1.40±0.04 ^b	1.50±0.00 ^d	1.35±0.03 ^c	1.30±0.00 ^c	1.22±0.03 ^d
Nimbecidine	1.95±0.19 ^a	1.62±0.05 ^b	1.40±0.04 ^b	1.60±0.04 ^b	1.92±0.06 ^b	1.95±0.06 ^b	1.70±0.04 ^b	1.52±0.03 ^b
Pyerin	1.97±0.10 ^a	1.52±0.03 ^{bc}	1.32±0.03 ^{bc}	1.50±0.04 ^b	1.67±0.03 ^c	1.87±0.09 ^{bc}	1.45±0.06 ^c	1.45±0.03 ^b
Pyneem	1.95±0.13 ^a	1.47±0.03 ^{bc}	1.35±0.05 ^{bc}	1.50±0.00 ^b	1.70±0.04 ^c	1.75±0.06 ^{bc}	1.35±0.05 ^c	1.35±0.03 ^b
MSD (0.05)	0.36	0.27	0.16	0.20	0.15	0.32	0.20	0.14
Without field margin vegetation								
Botanicals	First Spraying				Second Spraying			
	Pre-spray 1	7DAS	14DAS	21DAS	Pre spray 2	7DAS	14DAS	21DAS
Untreated control	2.52±0.06 ^a	2.40±0.04 ^a	2.57±0.03 ^a	2.47±0.05 ^a	2.67±0.05 ^a	3.12±0.13 ^a	2.95±0.10 ^a	2.67±0.08 ^a
Duduthrin	2.57±0.03 ^a	1.57±0.05 ^c	1.62±0.05 ^b	1.77±0.03 ^c	1.90±0.07 ^c	1.77±0.05 ^c	1.70±0.07 ^c	1.50±0.04 ^c
Nimbecidine	2.50±0.06 ^a	1.92±0.06 ^b	1.52±0.09 ^b	2.00±0.06 ^b	2.25±0.06 ^b	2.30±0.07 ^b	2.05±0.05 ^b	1.80±0.00 ^b
Pyerin	2.57±0.03 ^a	1.90±0.08 ^b	1.65±0.03 ^b	1.85±0.03 ^c	2.10±0.00 ^{bc}	2.17±0.05 ^b	1.80±0.06 ^c	1.65±0.03 ^{bc}
Pyneem	2.30±0.08 ^a	1.77±0.03 ^{bc}	1.62±0.06 ^b	1.87±0.03 ^{bc}	2.10±0.04 ^{bc}	2.02±0.08 ^{bc}	1.65±0.06 ^c	1.57±0.05 ^{bc}
MSD (0.05)	0.27	0.27	0.26	0.14	0.23	0.38	0.18	0.21

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

Table 3.7: Abundance (Mean±SE; n=4) of aphids in dolichos bean as affected by selected botanical insecticides and field margin vegetation (March-November 2020 cropping cycle).

With field margin vegetation								
Botanicals	First Spraying				Second Spraying			
	Pre-spray 1	7DAS	14DAS	21DAS	Pre-spray 2	7DAS	14DAS	21DAS
Untreated control	2.67±0.11 ^a	2.80±0.13 ^a	3.00±0.11 ^a	3.07±0.09 ^a	3.32±0.05 ^a	3.65±0.03 ^a	3.35±0.12 ^a	3.32±0.05 ^a
Duduthrin	2.72±0.05 ^a	1.70±0.09 ^b	1.85±0.03 ^b	2.17±0.09 ^b	2.12±0.09 ^b	2.10±0.00 ^b	1.90±0.04 ^c	1.62±0.05 ^b
Nimbecidine	2.80±0.11 ^a	2.07±0.10 ^b	2.12±0.11 ^b	2.32±0.03 ^b	2.22±0.05 ^b	2.20±0.11 ^b	2.17±0.03 ^b	1.80±0.07 ^b
Pyerin	2.67±0.09 ^a	1.82±0.03 ^b	1.90±0.04 ^b	2.10±0.03 ^b	2.27±0.03 ^b	2.27±0.06 ^b	2.07±0.03 ^{bc}	1.65±0.03 ^b
Pyneem	2.90±0.07 ^a	1.82±0.05 ^b	1.97±0.08 ^b	2.12±0.00 ^b	2.27±0.03 ^b	2.07±0.11 ^b	1.82±0.03 ^c	1.57±0.03 ^b
MSD (0.05)	0.26	0.42	0.38	0.26	0.25	0.35	0.25	0.23
Without field margin vegetation								
Botanicals	First Spraying				Second Spraying			
	Pre-spray 1	7DAS	14DAS	21DAS	Pre-spray 2	7DAS	14DAS	21DAS
Untreated control	3.65±0.06 ^a	3.57±0.06 ^a	3.65±0.05 ^a	3.60±0.10 ^a	4.10±0.07 ^a	4.30±0.07 ^a	4.12±0.06 ^a	3.77±0.06 ^a
Duduthrin	3.62±0.05 ^a	2.55±0.03 ^{bc}	2.37±0.05 ^b	2.47±0.06 ^b	2.57±0.11 ^b	2.47±0.03 ^b	2.25±0.05 ^{cd}	2.12±0.03 ^b
Nimbecidine	3.70±0.08 ^a	2.62±0.06 ^b	2.30±0.11 ^b	2.60±0.04 ^b	2.62±0.03 ^b	2.77±0.15 ^b	2.60±0.04 ^b	2.02±0.05 ^b
Pyerin	3.72±0.06 ^a	2.35±0.06 ^c	2.35±0.09 ^b	2.50±0.07 ^b	2.67±0.03 ^b	2.60±0.10 ^b	2.40±0.04 ^c	1.95±0.03 ^b
Pyneem	3.70±0.07 ^a	2.50±0.06 ^{bc}	2.37±0.03 ^b	2.52±0.03 ^b	2.47±0.03 ^b	2.47±0.03 ^b	2.17±0.05 ^d	1.92±0.06 ^b
MSD (0.05)	0.31	0.23	0.30	0.27	0.24	0.42	0.16	0.22

Means in a column followed by the same letter are not significantly different using Tukey's HSD test at $P < 0.05$. DAS – days after spraying; Pre-spray 1 = spraying at 42 days after planting; Pre-spray 2 = Spraying at 70 days after planting; MSD = Minimum significant difference. Aphid abundance index: 1 = no aphids; 2 = A few scattered aphids; 3 = A few small colonies; 4 = Several small colonies; 5 = Large isolated colonies; and 6 = Large continuous colonies.

3.3.3 Effect of botanical insecticides on damage severity

Across cropping cycles, damage severity differed significantly ($P < 0.0001$) with the highest damage severity observed in March-November 2020 (2.57) as compared to May-December 2019 (1.88). In the 2019 cropping cycle, botanical insecticide significantly influenced aphid damage severity ($P < 0.0001$). At 21 DAS for the first spraying, the highest damage score was observed in untreated plots (1.87) with the lowest score in plots treated with Duduthrin (1.35) which was not different from Pyerin (1.55), Pyeneem (1.60) and Nimbecidine (1.70). Similarly, at 21 DAS for the second spray, the highest damage score was observed in untreated plots (2.22) with the lowest score in plots treated with Duduthrin (1.35) and Pyeneem (1.65) which were not different from Pyerin (1.42) and Nimbecidine (1.47) (Table 3.8). In the absence of field margin vegetation, untreated plots had high damage severity with the highest damage score of 2.82 at 70 days after planting. During the first spray at 21 DAS, the lowest aphid damage severity was observed in plots treated with Pyeneem (1.85) and Duduthrin (1.85) which were not different from Pyerin (1.90) and Nimbecidine (2.10). Similar results were observed during the second spray at 21 DAS with the lowest damage score in plots treated with Pyeneem and Pyerin (1.65) that were not different from Duduthrin (1.67) and Nimbecidine (1.72) (Table 3.8).

In the 2020 cropping cycle, botanical insecticides significantly affected damage severity ($P < 0.0001$). In the presence of field margin vegetation, at 21 DAS after the first spray, the highest damage score was observed in untreated plots at 2.90 and lowest in plots treated with Pyeneem (2.15) and Pyerin (2.15) which were not significantly different from Duduthrin (2.17) and Nimbecidine (2.37). At 21 DAS for the second spray, untreated plots had the highest damage score of 3.00 compared to the plot that was treated with Pyeneem (1.57), Pyerin (1.70), and Nimbecidine (1.80) and Duduthrin (1.67). In the absence, untreated plots had the highest damage severity throughout the growing cycle. At 21 DAS for the first spray, among the botanical insecticides, Pyerin-treated plots had the lowest damage score of 2.52 which was not significantly different from Pyeneem (2.65), Nimbecidine (2.87) and Duduthrin (2.60). Similar

results were observed at 21 DAS for the second spray where Pyeneem (1.85) and Pyerin (1.97) treated plots had the lowest damage compared to Nimbecidine (2.15) and Duduthrin (2.07) (Table 3.9).

Table 3.8: Severity (Mean±SE; n=4) of damage caused by aphids on dolichos bean as affected by selected botanical insecticides and field margin vegetation (May-December 2019 cropping cycle).

With field margin vegetation								
Botanicals	First Spraying				Second Spraying			
	Pre-spray 1	7DAS	14DAS	21DAS	Pre-spray 2	7DAS	14DAS	21DAS
Untreated control	1.35±0.03 ^a	1.92±0.09 ^a	1.97±0.03 ^a	1.87±0.14 ^a	2.35±0.03 ^a	2.27±0.03 ^a	2.35±0.03 ^a	2.22±0.03 ^a
Duduthrin	1.37±0.05 ^a	1.35±0.03 ^b	1.37±0.03 ^c	1.50±0.07 ^b	1.65±0.09 ^d	1.75±0.06 ^b	1.55±0.06 ^{cd}	1.35±0.04 ^b
Nimbecidine	1.35±0.03 ^a	1.57±0.05 ^b	1.75±0.15 ^{ab}	1.70±0.09 ^{ab}	2.05±0.03 ^b	1.80±0.04 ^b	1.82±0.06 ^b	1.47±0.03 ^b
Pyerin	1.32±0.03 ^a	1.42±0.06 ^b	1.47±0.03 ^{bc}	1.55±0.03 ^{ab}	1.90±0.04 ^{bc}	1.82±0.05 ^b	1.72±0.03 ^{bc}	1.42±0.03 ^b
Pyneem	1.35±0.03 ^a	1.45±0.03 ^b	1.42±0.03 ^c	1.60±0.04 ^{ab}	1.70±0.07 ^{cd}	1.67±0.09 ^b	1.37±0.11 ^c	1.35±0.03 ^b
MSD (0.05)	0.15	0.25	0.29	0.34	0.23	0.27	0.25	0.14
Without field margin vegetation								
Botanicals	First spraying				Second spraying			
	Pre-spray 1	7DAS	14DAS	21DAS	Pre-spray 2	7DAS	14DAS	21DAS
Untreated control	1.67±0.00 ^a	2.30±0.06 ^a	2.37±0.05 ^a	2.35±0.18 ^a	2.82±0.05 ^a	2.75±0.06 ^a	2.77±0.05 ^a	2.57±0.05 ^a
Duduthrin	1.72±0.05 ^a	1.67±0.05 ^c	1.77±0.09 ^b	1.85±0.05 ^b	2.07±0.05 ^c	2.07±0.05 ^b	1.82±0.08 ^b	1.67±0.08 ^b
Nimbecidine	1.72±0.03 ^a	2.00±0.04 ^b	1.85±0.09 ^b	2.10±0.16 ^{ab}	2.47±0.05 ^b	2.10±0.08 ^b	2.05±0.05 ^b	1.72±0.03 ^b
Pyerin	1.82±0.10 ^a	1.82±0.09 ^{bc}	1.72±0.06 ^b	1.90±0.04 ^{ab}	2.40±0.04 ^b	2.12±0.03 ^b	2.00±0.04 ^{bc}	1.65±0.03 ^b
Pyneem	1.72±0.03 ^a	1.90±0.04 ^{bc}	1.77±0.09 ^b	1.85±0.05 ^b	2.00±0.05 ^c	1.95±0.06 ^b	1.67±0.09 ^c	1.65±0.05 ^b
MSD (0.05)	0.29	0.27	0.31	0.47	0.20	0.28	0.26	0.20

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

Table 3.9: Severity (Mean $\pm$ SE; n=4) of damage caused by aphids on dolichos bean as affected by selected botanical insecticides and field margin vegetation (March-November 2020 cropping cycle).

With field margin vegetation								
Botanicals	First spraying				Second spraying			
	Pre-spray 1	7DAS	14DAS	21DAS	Pre-spray 2	7DAS	14DAS	21DAS
Untreated control	1.62 $\pm$ 0.03 ^a	2.15 $\pm$ 0.13 ^a	3.02 $\pm$ 0.08 ^a	2.90 $\pm$ 0.24 ^a	3.32 $\pm$ 0.05 ^a	3.37 $\pm$ 0.03 ^a	3.22 $\pm$ 0.05 ^a	3.00 $\pm$ 0.06 ^a
Duduthrin	1.60 $\pm$ 0.06 ^a	1.70 $\pm$ 0.09 ^b	1.87 $\pm$ 0.03 ^b	2.17 $\pm$ 0.11 ^b	2.37 $\pm$ 0.03 ^b	2.15 $\pm$ 0.03 ^b	2.00 $\pm$ 0.10 ^{bc}	1.67 $\pm$ 0.06 ^b
Nimbecidine	1.65 $\pm$ 0.06 ^a	1.75 $\pm$ 0.03 ^b	2.30 $\pm$ 0.26 ^b	2.37 $\pm$ 0.18 ^{ab}	2.17 $\pm$ 0.09 ^{bc}	2.20 $\pm$ 0.00 ^b	2.25 $\pm$ 0.06 ^b	1.80 $\pm$ 0.04 ^b
Pyerin	1.77 $\pm$ 0.09 ^a	1.67 $\pm$ 0.03 ^b	1.85 $\pm$ 0.10 ^b	2.15 $\pm$ 0.03 ^b	2.15 $\pm$ 0.03 ^{bc}	2.22 $\pm$ 0.06 ^b	2.15 $\pm$ 0.10 ^b	1.70 $\pm$ 0.04 ^b
Pyneem	1.57 $\pm$ 0.05 ^a	1.67 $\pm$ 0.06 ^b	2.00 $\pm$ 0.16 ^b	2.15 $\pm$ 0.09 ^b	2.10 $\pm$ 0.07 ^c	2.07 $\pm$ 0.13 ^b	1.77 $\pm$ 0.05 ^c	1.57 $\pm$ 0.04 ^b
MSD (0.05)	0.26	0.36	0.56	0.55	0.24	0.30	0.37	0.24
Without field margin vegetation								
Botanicals	First spraying				Second spraying			
	Pre-spray 1	7DAS	14DAS	21DAS	Pre-spray 2	7DAS	14DAS	21DAS
Untreated control	2.02 $\pm$ 0.11 ^a	2.50 $\pm$ 0.04 ^a	3.62 $\pm$ 0.06 ^a	3.30 $\pm$ 0.27 ^a	3.95 $\pm$ 0.15 ^a	4.17 $\pm$ 0.05 ^a	3.95 $\pm$ 0.06 ^a	3.62 $\pm$ 0.08 ^a
Duduthrin	1.87 $\pm$ 0.09 ^a	2.35 $\pm$ 0.03 ^{ab}	2.42 $\pm$ 0.05 ^{bc}	2.60 $\pm$ 0.14 ^b	2.75 $\pm$ 0.03 ^{bc}	2.52 $\pm$ 0.03 ^b	2.35 $\pm$ 0.05 ^{bc}	2.07 $\pm$ 0.06 ^b
Nimbecidine	1.97 $\pm$ 0.08 ^a	2.30 $\pm$ 0.07 ^{ab}	2.72 $\pm$ 0.16 ^b	2.87 $\pm$ 0.21 ^{ab}	2.80 $\pm$ 0.04 ^b	2.50 $\pm$ 0.00 ^b	2.47 $\pm$ 0.05 ^b	2.15 $\pm$ 0.06 ^b
Pyerin	2.10 $\pm$ 0.11 ^a	2.25 $\pm$ 0.05 ^b	2.27 $\pm$ 0.03 ^c	2.52 $\pm$ 0.03 ^b	2.55 $\pm$ 0.03 ^{bc}	2.52 $\pm$ 0.05 ^b	2.42 $\pm$ 0.05 ^{bc}	1.97 $\pm$ 0.05 ^{bc}
Pyneem	1.87 $\pm$ 0.08 ^a	2.20 $\pm$ 0.07 ^b	2.42 $\pm$ 0.05 ^{bc}	2.65 $\pm$ 0.09 ^b	2.45 $\pm$ 0.03 ^c	2.47 $\pm$ 0.04 ^b	2.20 $\pm$ 0.04 ^c	1.85 $\pm$ 0.03 ^c
MSD (0.05)	0.41	0.23	0.38	0.59	0.32	0.18	0.23	0.22

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

DAS – days after spraying; Pre-spray 1 = spraying at 42 days after planting; Pre-spray 2= Spraying at 70 days after planting; MSD= Minimum significant difference. Damage severity: 1= no damage, 2=light damage < 25 %, 3= damage 26 % - 50 %, 4= damage 51 % - 75 % yellowing of lower leaves; 5= damage >75 % yellow and severely curled.

3.3.4 Effect of botanical insecticides on aphid incidence

Results showed that botanical insecticides significantly ($P<0.0001$) influenced aphid incidence across the two cropping cycles. The highest aphid percent incidence was observed in the untreated plots (62.70%) as compared to Pyerin (49.38%), Pyeneem (49.30%), Nimbecidine (52.15%) and Duduthrin (45.86%). During the 2019 cropping cycle, results revealed that botanical insecticides significantly ($P<0.0001$) affected aphid incidence. In the presence of field margin vegetation at 7 DAS for the first spraying, the percent incidence was reduced in the treated plots as compared to the untreated control. Among the botanical insecticides, Pyeneem treated plots had the lowest percent incidence (40.00%) which was not significantly ($P=0.05$) different from Pyerin (41.25%), Nimbecidine (41.25%) and Duduthrin (38.75%). At 21 DAS for the second spraying, the lowest score was observed in plots treated with Pyeneem (30.00%) which was not different from Pyerin (32.50%), Nimbecidine (35.00%) and Duduthrin (28.75%). In the absence of field margin vegetation at 21 DAS for the second spray, the lowest aphid incidence was observed in plots treated with Pyeneem (36.25%) which was not different from Pyerin (41.25%), Nimbecidine (45.00%) and Duduthrin (37.50%) (Table 3.10).

In the 2020 cropping cycle, botanical insecticides significantly ($P<0.0001$) influenced aphid incidence. In the presence of field margin vegetation at 21 DAS for the first spray, the highest incidence score was observed in untreated plots (55.00%). Among the botanical insecticides, the lowest score was observed in Pyerin (46.25%) which was not significantly ($P=0.05$) different from Pyeneem (47.50%) and Duduthrin (47.50%). At 21 DAS for the second spray, the lowest aphid percent incidence was observed in plots treated with Pyeneem (32.50%) and Duduthrin (32.50%) which were not different from Pyerin (36.25%) and Nimbecidine (38.75%). Similarly, in the absence of field margin vegetation, the untreated plots had the highest aphid percent incidence compared to treated plots. At 21 DAS for the first spray, the lowest incidence score was observed in plots treated with Pyerin (53.75%) which was not different from Pyeneem (57.50%) and Duduthrin (57.50%) compared to untreated control (73.75%). At 21 DAS for the second spray, the lowest aphid percent incidence was

observed in plots treated with Pyeneem (41.25%) and Duduthrin (42.50%) which were not different from Pyerin (45.00%) (Table 3.11).

Table 3.10: Incidence (Mean $\pm$ SE; n=4) of aphids in dolichos bean as affected by selected botanical insecticides and field margin vegetation (May-December 2019cropping cycle).

With field margin vegetation								
Botanicals	First spraying				Second spraying			
	Pre-spray 1	7DAS	14DAS	21DAS	Pre-spray 2	7DAS	14DAS	21DAS
Untreated control	53.75 $\pm$ 2.39 ^b	55.00 $\pm$ 2.04 ^a	51.25 $\pm$ 1.25 ^a	56.25 $\pm$ 2.39 ^a	55.00 $\pm$ 3.54 ^a	62.50 $\pm$ 2.50 ^a	55.00 $\pm$ 2.04 ^a	50.00 $\pm$ 2.04 ^a
Duduthrin	56.25 $\pm$ 3.75 ^{ab}	38.75 $\pm$ 1.25 ^b	27.50 $\pm$ 1.44 ^b	40.00 $\pm$ 0.00 ^c	41.25 $\pm$ 1.25 ^b	40.00 $\pm$ 2.04 ^c	31.25 $\pm$ 1.25 ^c	28.75 $\pm$ 1.25 ^b
Nimbecidine	56.25 $\pm$ 3.15 ^{ab}	41.25 $\pm$ 1.25 ^b	38.75 $\pm$ 4.27 ^b	50.00 $\pm$ 2.04 ^{ab}	51.25 $\pm$ 1.25 ^a	50.00 $\pm$ 0.00 ^b	41.25 $\pm$ 1.25 ^b	35.00 $\pm$ 2.04 ^b
Pyerin	56.25 $\pm$ 3.15 ^{ab}	41.25 $\pm$ 1.25 ^b	38.75 $\pm$ 2.39 ^b	42.50 $\pm$ 1.44 ^{bc}	51.25 $\pm$ 1.25 ^a	47.50 $\pm$ 1.44 ^{bc}	35.00 $\pm$ 2.04 ^{bc}	32.50 $\pm$ 1.44 ^b
Pyneem	67.50 $\pm$ 4.33 ^a	40.00 $\pm$ 0.00 ^b	37.50 $\pm$ 1.44 ^b	45.00 $\pm$ 0.00 ^{bc}	47.50 $\pm$ 1.44 ^{ab}	46.25 $\pm$ 1.25 ^{bc}	33.75 $\pm$ 1.25 ^c	30.00 $\pm$ 0.00 ^b
MSD (0.05)	12.51	6.25	11.86	7.69	8.90	7.83	7.20	6.50
Without field margin vegetation								
Botanicals	First spraying				Second spraying			
	Pre-spray 1	7DAS	14DAS	21DAS	Pre-spray 2	7DAS	14DAS	21DAS
Untreated control	62.50 $\pm$ 1.44 ^a	62.50 $\pm$ 2.50 ^a	60.00 $\pm$ 2.04 ^a	67.50 $\pm$ 1.44 ^a	67.50 $\pm$ 3.23 ^a	70.00 $\pm$ 2.04 ^a	66.25 $\pm$ 1.25 ^a	61.25 $\pm$ 2.39 ^a
Duduthrin	63.75 $\pm$ 1.25 ^a	50.00 $\pm$ 2.04 ^b	42.50 $\pm$ 1.44 ^a	43.75 $\pm$ 5.15 ^b	43.75 $\pm$ 1.25 ^c	48.75 $\pm$ 1.25 ^c	40.00 $\pm$ 2.89 ^c	37.50 $\pm$ 3.23 ^b
Nimbecidine	62.50 $\pm$ 1.44 ^a	53.75 $\pm$ 1.25 ^{ab}	42.50 $\pm$ 1.22 ^a	56.25 $\pm$ 3.15 ^{ab}	61.25 $\pm$ 1.25 ^{ab}	56.25 $\pm$ 1.25 ^b	51.25 $\pm$ 1.25 ^b	45.00 $\pm$ 2.04 ^b
Pyerin	63.75 $\pm$ 2.39 ^a	52.50 $\pm$ 2.50 ^b	48.75 $\pm$ 2.39 ^a	51.25 $\pm$ 1.25 ^b	57.50 $\pm$ 1.44 ^b	53.75 $\pm$ 1.25 ^{bc}	46.25 $\pm$ 1.25 ^{bc}	41.25 $\pm$ 1.25 ^b
Pyneem	63.75 $\pm$ 1.25 ^a	55.00 $\pm$ 0.00 ^{ab}	52.50 $\pm$ 1.44 ^a	51.25 $\pm$ 1.25 ^b	55.00 $\pm$ 2.04 ^b	55.00 $\pm$ 2.04 ^{bc}	43.75 $\pm$ 1.25 ^c	36.25 $\pm$ 1.25 ^b
MSD (0.05)	7.48	9.53	17.81	14.10	9.81	7.48	8.48	9.37

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

Table 3. 11: Incidence (Mean $\pm$ SE; n=4) of aphids in dolichos bean as affected by selected botanical insecticides and field margin vegetation (March-November 2020cropping cycle).

With field margin vegetation								
Botanicals	First spraying				Second spraying			
	Pre-spray 1	7DAS	14DAS	21DAS	Pre-spray 2	7DAS	14DAS	21DAS
Untreated control	60.00 $\pm$ 0.00 ^a	60.00 $\pm$ 2.04 ^a	62.50 $\pm$ 2.50 ^a	55.00 $\pm$ 2.04 ^a	63.75 $\pm$ 2.39 ^a	66.25 $\pm$ 2.39 ^a	56.25 $\pm$ 3.15 ^a	53.75 $\pm$ 1.25 ^a
Duduthrin	61.25 $\pm$ 2.39 ^a	46.25 $\pm$ 1.25 ^b	36.25 $\pm$ 3.15 ^c	47.50 $\pm$ 1.44 ^{bc}	48.75 $\pm$ 1.25 ^c	45.00 $\pm$ 2.04 ^b	38.75 $\pm$ 1.25 ^b	32.50 $\pm$ 1.44 ^b
Nimbecidine	55.00 $\pm$ 2.89 ^a	46.25 $\pm$ 1.25 ^b	50.00 $\pm$ 3.54 ^b	53.75 $\pm$ 1.25 ^{ab}	55.00 $\pm$ 0.00 ^b	51.25 $\pm$ 1.25 ^b	43.75 $\pm$ 1.25 ^b	38.75 $\pm$ 1.25 ^b
Pyerin	55.00 $\pm$ 3.54 ^a	42.50 $\pm$ 1.44 ^b	42.50 $\pm$ 1.44 ^{bc}	46.25 $\pm$ 1.25 ^c	53.75 $\pm$ 1.25 ^{bc}	50.00 $\pm$ 0.00 ^b	37.50 $\pm$ 1.44 ^b	36.25 $\pm$ 3.15 ^b
Pyneem	55.00 $\pm$ 2.09 ^a	45.00 $\pm$ 0.00 ^b	43.75 $\pm$ 1.25 ^{bc}	47.50 $\pm$ 1.44 ^{bc}	51.25 $\pm$ 1.25 ^{bc}	51.25 $\pm$ 1.25 ^b	35.00 $\pm$ 2.89 ^b	32.50 $\pm$ 1.44 ^b
MSD (0.05)	13.09	5.14	9.37	6.42	5.03	7.48	10.02	7.02
Without field margin vegetation								
Botanicals	First Spraying				Second Spraying			
	Pre-spray 1	7DAS	14DAS	21DAS	Pre-spray 2	7DAS	14DAS	21DAS
Untreated control	70.00 $\pm$ 0.00 ^a	70.00 $\pm$ 2.04 ^a	73.75 $\pm$ 1.25 ^a	73.75 $\pm$ 1.25 ^a	75.00 $\pm$ 2.04 ^a	76.25 $\pm$ 1.25 ^a	68.75 $\pm$ 1.25 ^a	65.00 $\pm$ 2.04 ^a
Duduthrin	70.00 $\pm$ 0.00 ^a	55.00 $\pm$ 0.00 ^b	52.50 $\pm$ 1.44 ^b	57.50 $\pm$ 1.44 ^c	56.25 $\pm$ 1.25 ^c	55.00 $\pm$ 2.04 ^c	48.75 $\pm$ 1.25 ^c	42.50 $\pm$ 1.44 ^c
Nimbecidine	70.00 $\pm$ 0.00 ^a	56.25 $\pm$ 1.25 ^b	57.50 $\pm$ 1.25 ^b	66.25 $\pm$ 1.25 ^b	65.00 $\pm$ 0.00 ^b	62.50 $\pm$ 1.44 ^b	55.00 $\pm$ 0.00 ^b	50.00 $\pm$ 0.00 ^b
Pyerin	66.25 $\pm$ 1.25 ^a	56.25 $\pm$ 2.39 ^b	56.25 $\pm$ 2.39 ^b	53.75 $\pm$ 2.39 ^c	63.75 $\pm$ 1.25 ^b	63.75 $\pm$ 1.25 ^b	51.25 $\pm$ 1.25 ^{bc}	45.00 $\pm$ 2.04 ^{bc}
Pyneem	70.00 $\pm$ 0.00 ^a	60.00 $\pm$ 0.00 ^b	57.50 $\pm$ 1.44 ^b	57.50 $\pm$ 1.44 ^c	61.25 $\pm$ 1.25 ^{bc}	61.25 $\pm$ 1.25 ^{bc}	47.50 $\pm$ 1.44 ^c	41.25 $\pm$ 1.25 ^c
MSD (0.05)	2.51	6.25	9.14	7.20	6.58	6.74	5.34	6.17

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

DAS – days after spraying; Pre-spray 1 = spraying at 42 days after planting; Pre-spray 2= Spraying at 70 days after planting; MSD= Minimum significant difference.

3.3.5 Effect of cropping cycle and field margin vegetation on the abundance of natural enemies (parasitic wasps, tachinid flies and ladybird beetles).

Arthropods collected were first grouped as either predator or parasitoid which are the main categories of bean aphid natural enemies. From the initial sorting, a total of 6,808 of the natural enemies were collected during the two cropping cycles. The major groups identified were parasitic wasps (Braconidae and Ichneumonidae) at 40%, tachinid flies (Tachinidae) at 43% and ladybird beetles (Coccinellidae) at 17% (Figure 3.3). There was a significant cropping cycle effect on the abundance of parasitic wasps ($P<0.0001$), tachinid flies ($P<0.0001$), and ladybird beetles ($P<0.0001$) (Appendix C). During the March-November 2020 cropping cycle, more natural enemies (70%) were collected than during the May-December 2019 cropping cycle (30%). Between the two cropping cycles, tachinid flies and parasitic wasps were the most abundant (43% and 40%, respectively). In plots with field margin vegetation, more natural enemies were collected as compared to plots with no field margin vegetation which was consistent in the two cropping cycles (Table 3.12). Natural enemies significantly differed in the trapping area (crop center and within field margin vegetation) for parasitic wasps ($P<0.0001$), tachinid flies ($P<0.0001$) and ladybird beetles ($P<0.0001$). More natural enemies were collected at the crop center compared to field margin vegetation which was consistent across the two cropping cycles for parasitic wasps, tachinid flies and ladybird beetles (Table 3.12).

Table 3.12: Number (Mean $\pm$ SE; n=160) of parasitic wasps, tachinid flies and ladybird beetles as influenced by presence or absence of field margin vegetation and trapping area.

	May-December 2019 Cycle			March-November 2020 Cycle		
	Parasitic Wasps	Tachinid flies	Ladybird beetles	Parasitic Wasps	Tachinid flies	Ladybird beetles
FMV						
Present	2.21 $\pm$ 0.09 ^a	2.48 $\pm$ 0.12 ^a	2.49 $\pm$ 0.11 ^a	7.76 $\pm$ 0.33 ^a	8.35 $\pm$ 0.32 ^a	1.37 $\pm$ 0.07 ^b
Absent	1.71 $\pm$ 0.08 ^b	1.91 $\pm$ 0.10 ^b	1.79 $\pm$ 0.08 ^b	5.45 $\pm$ 0.37 ^b	5.43 $\pm$ 0.34 ^b	1.60 $\pm$ 0.14 ^a
MSD (0.05)	0.21	0.25	0.22	0.72	0.75	0.41
Trapping Area						
Crop center	2.14 $\pm$ 0.09a	2.05 $\pm$ 0.10b	2.03 $\pm$ 0.09a	10.12 $\pm$ 0.27a	9.96 $\pm$ 0.29a	2.06 $\pm$ 0.12a
Within FMV	1.77 $\pm$ 0.08b	2.33 $\pm$ 0.11a	2.25 $\pm$ 0.10b	3.08 $\pm$ 0.17b	3.83 $\pm$ 0.22b	0.90 $\pm$ 0.06b
MSD (0.05)	0.21	0.24	0.22	0.39	0.41	0.22

Means in a column followed by the same letters are not significantly different using Tukey's HSD test at $P < 0.05$; FMV = Field margin vegetation; MSD= Minimum significant difference.

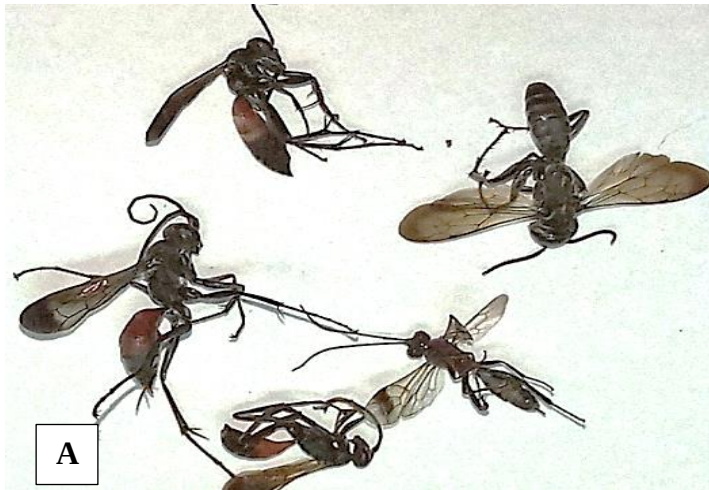


Figure 3.3: Collected natural enemies of bean aphid: Parasitic wasps (A), Tachinid flies (B) and ladybird beetles (C).

3.3.6 Effect of botanical insecticides on the abundance of parasitic wasps

Across the cropping cycles, parasitic wasp abundance differed significantly ($P < 0.0001$) with high abundance observed during the March-November 2020 cropping cycle (6.6) as compared to the May-December 2019 (1.95). Results of the 2019 cropping cycle revealed that botanical insecticides significantly ($P < 0.0001$) influenced the abundance of parasitic wasps. In the presence of field margin vegetation, a high abundance of parasitic wasps was observed at 42 DAP (av. 2.1) as compared to plots that had no field margin vegetation (av. 1.72). Both in the presence and absence of field margin vegetation, the abundance of parasitic wasps was lowest in plots treated with Dududthrin at 7-days post- the second spray. In the presence of field margin vegetation, the lowest parasitic wasp abundance (0.9) was observed in plots that were treated with Duduthrin compared to Pyeneem (1.6), Pyerin (1.6) and Nimbecidine (2.8) at 7-days post-second spray (Figure 3.4). In the absence of field margin vegetation, the lowest abundance of parasitic wasps was observed in plots treated with Duduthrin (1.0) compared to botanical insecticides Pyeneem (1.8), Pyerin (1.5) and Nimbecidine (2.1) at 7-days post-second spray (Figure 3.4).

In the March-November 2020 cropping cycle, botanical insecticide application significantly ($P < 0.0001$) affected the abundance of parasitic wasps. As compared to treated plots, untreated plots had a higher abundance of parasitic wasps both in the presence and absence of field margin vegetation. In the presence of field margin vegetation, parasitic wasps' abundance of 5.1 was observed in plots treated with Duduthrin compared to Pyeneem (6.7) and untreated (10.6) at 7-days post first spray. In the absence of field margin vegetation, the lowest parasitic wasp abundance of 2.6 was observed in plots treated with Duduthrin compared to botanical insecticides, Pyeneem (4.1), Pyerin (4.0) and Nimbecidine (5.1) at 7-days post first spray and untreated control (5.6) (Figure 3.5).

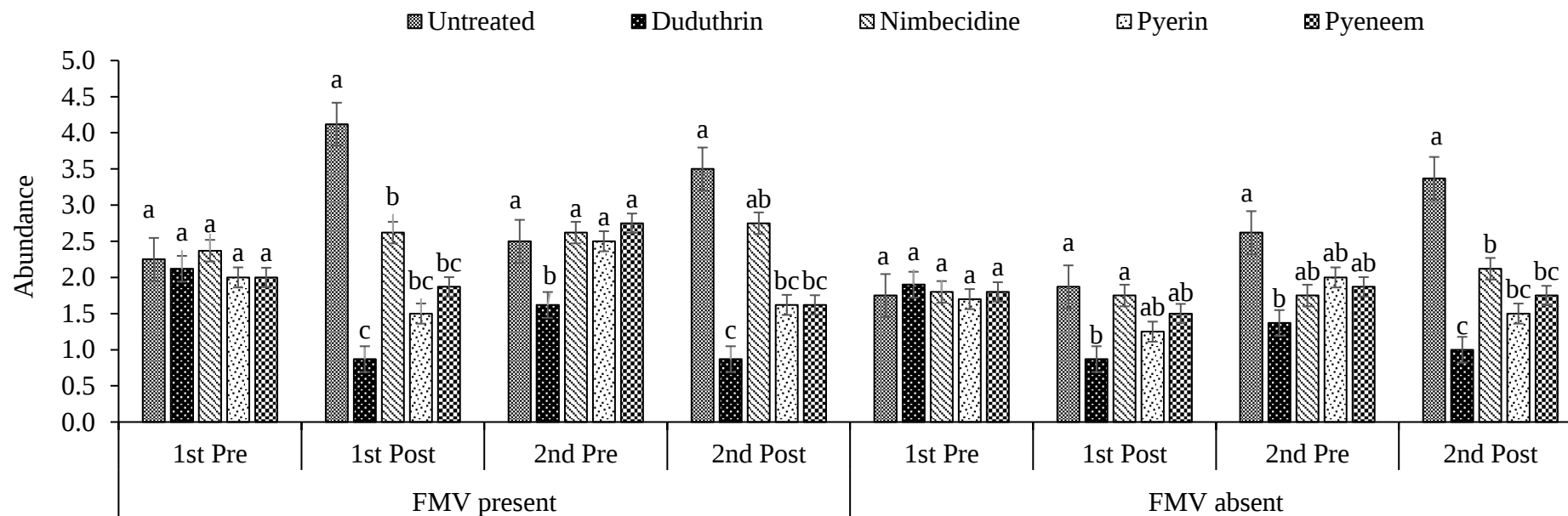


Figure 3.4: Abundance (Mean±SE; n=8) of parasitic wasps in dolichos bean pre-and post-botanical insecticides application in the presence or absence of field margin vegetation (May-December 2019 cropping cycle). FMV = Field margin vegetation; Pre-spray 1 = spraying at 42 days after planting; Pre-spray 2= Spraying at 70 days after planting. Bars with the same letters each time before and after spraying are not significantly different using Tukey's HSD test at $P < 0.05$.

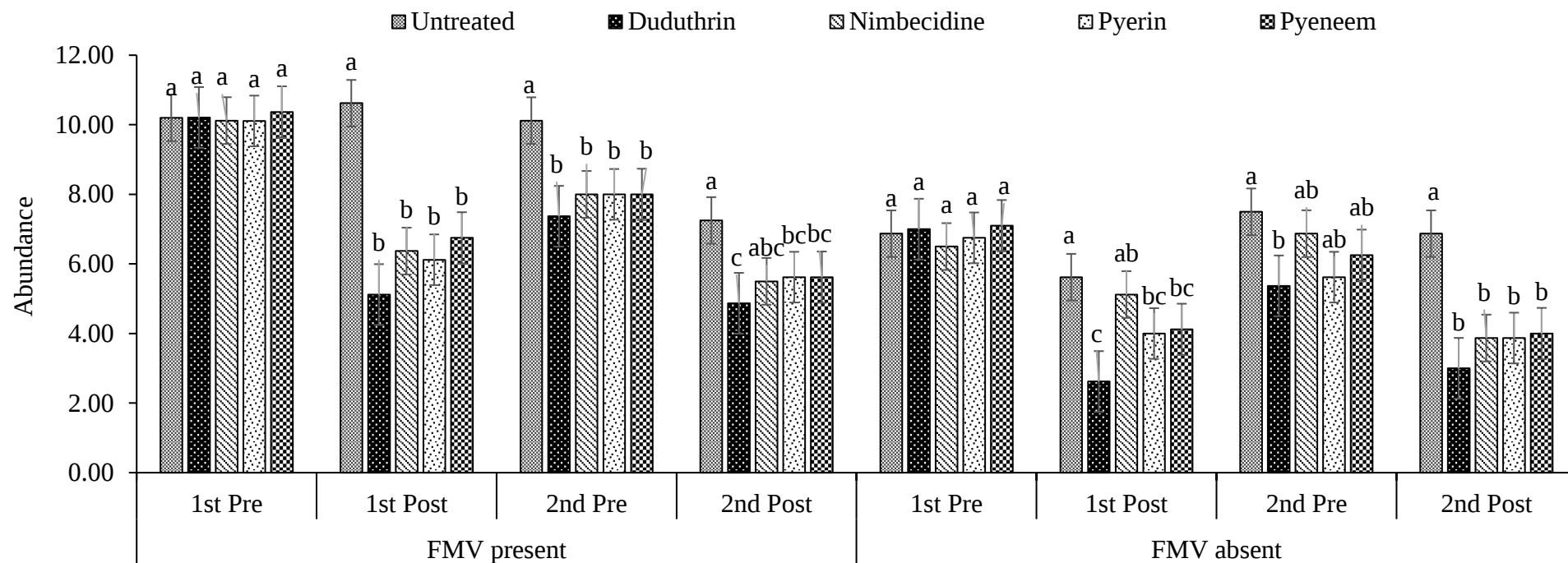


Figure 3. 5: Abundance (Mean±SE; n=8) of parasitic wasps in dolichos bean pre-and post-botanical insecticides application in the presence or absence of field margin vegetation (March-Novemeber 2020 cropping cycle). FMV = Field margin vegetation; Pre-spray 1 = spraying at 42 days after planting; Pre-spray 2= Spraying at 70 days after planting. Bars with the same letters each time before and after spraying are not significantly different using Tukey’s HSD test at $P < 0.05$.

3.3.7 Effect of botanical insecticides on the abundance of ladybird beetles

Results revealed that the cropping cycle significantly ($P < 0.0001$) affected the abundance of ladybird beetles. During the May-December 2019 cropping cycle, ladybird beetle abundance was high (2.1) as compared to March-November 2020 (1.5). In the 2019 cropping cycle, botanical insecticide application significantly ($P < 0.0001$) affected the abundance of ladybird beetles. In the presence of field margin vegetation, a high abundance of ladybird beetles (av. 3.0) was observed at 42 DAP as compared to plots that had no field margin vegetation (av. 1.9) (Figure 3.6). Generally, there was a higher abundance of ladybird beetles in untreated plots in the presence of field margin vegetation as compared to plots that had no field margin vegetation. In treated plots, Duduthrin had the lowest abundance of ladybird beetles both in the presence and absence of field margin vegetation at 7-days post-second spray (Figure 3.6).

During the 2020 cropping cycle, botanical insecticides significantly ($P = 0.0005$) affected the abundance of ladybird beetles. There was no significant interaction effect between field margin vegetation and botanical insecticides ($P = 0.42$). At 42 DAP, a higher abundance of ladybird beetles was observed in plots with no field margin vegetation (av. 2.9) as compared to plots that were surrounded by field margin vegetation (av. 1.9). However, in the absence of field margin vegetation, abundance was reduced in the treated plots with the lowest abundance observed in Duduthrin (0.2) at 7-days post the second spray as compared to plots treated with Duduthrin in the presence of field margin vegetation during the same period (Figure 3.7). In the presence of field margin vegetation, Nimbecidine treated plots had a higher abundance of 1.0 compared to plots treated with Pyeneem (0.6), Pyerin (0.6) and Duduthrin (0.5) at 7-days post-second spray. Similarly, in the absence of field margin vegetation Nimbecidine treated plots had a higher abundance of 1.0 as compared to plots treated with Pyeneem (0.6), Pyerin (0.5) and Duduthrin (0.3) at 7-days post-second spray (Figure 3.7)

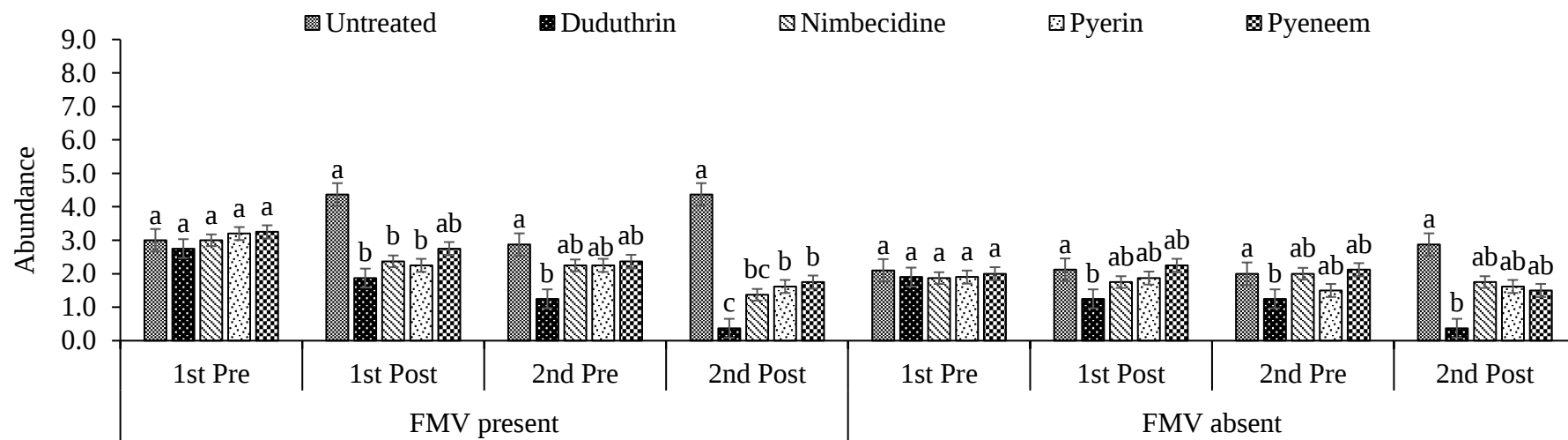


Figure 3. 6: Abundance (Mean±SE; n=8) of ladybird beetles in dolichos bean pre-and post-botanical insecticides application in the presence or absence of field margin vegetation (May-December 2019 cropping cycle). FMV = Field margin vegetation; Pre-spray 1 = spraying at 42 days after planting; Pre-spray 2= Spraying at 70 days after planting Bars with the same letters each time before and after spraying are not significantly different using Tukey's HSD test at $P < 0.05$.

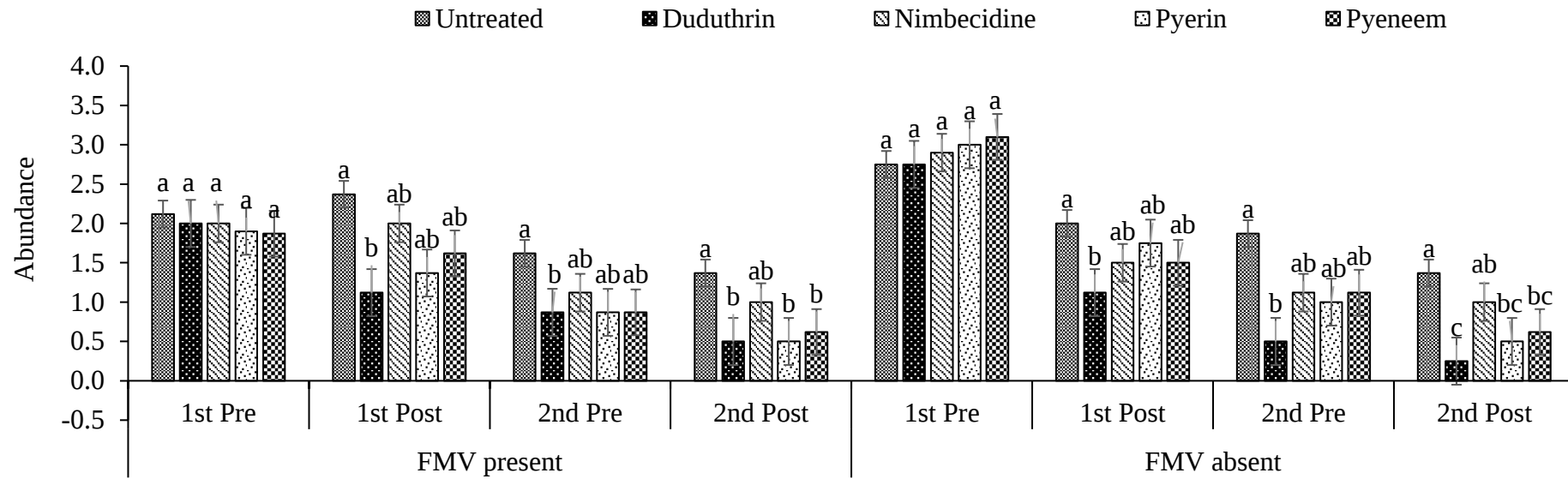


Figure 3. 7: Abundance (Mean±SE; n=8) of ladybird beetles in dolichos bean pre-and post-botanical insecticides application in the presence or absence of field margin vegetation (March-November 2020 cropping cycle). FMV = Field margin vegetation; Pre-spray 1 = spraying at 42 days after planting; Pre-spray 2= Spraying at 70 days after planting. Bars with the same letters each time before and after spraying are not significantly different using Tukey's HSD test at $P < 0.05$.

3.3.8 Effect of botanical insecticides on the abundance of tachinid flies

Results indicated that the cropping cycle significantly ($P<0.0001$) affected the abundance of tachinid flies. The highest abundance of tachinid flies was in March-November 2020 (av. 6.8) as compared to May-December 2019 (av. 2.1). In the 2019 cropping cycle, botanical insecticide application significantly ($P<0.0001$) affected the abundance of tachinid flies. In the presence of field margin vegetation high abundance of tachinid flies (av. 3.6) was observed at 42 DAP as compared to plots that had no field margin vegetation (av. 2.2) during the same period (Figure 3.8). The abundance of tachinid flies was reduced in treated plots 7-days post-first and second sprays both in the presence and absence of field margin vegetation. In the presence of field margin vegetation, the lowest tachinid fly abundance was observed in plots treated with Duduthrin (1.0) which was statistically different ($P=0.05$) from the three botanical insecticides; Pyeneem (1.8), Pyerin (1.6) and Nimbecidine (2.1) at 7-days post-second spray. In the absence of field margin vegetation, the lowest abundance was observed in plots treated with Duduthrin (0.5) which was statistically different ($P=0.05$) from Pyeneem (2.5), Pyerin (2.0) and Nimbecidine (1.8) (Figure 3.8).

In the 2020 cropping cycle, botanical insecticide application significantly ($P<0.0001$) affected the abundance of tachinid flies. As compared to treated plots, untreated plots had a high abundance of tachinid flies both in the presence and absence of field margin vegetation 7-days post first and second sprays. In the presence of field margin vegetation, an abundance score of 4.0 was observed in plots treated with Duduthrin which was statistically different ($P=0.05$) from Pyerin (5.6) and Nimbecidine (5.6) at 7-days post-second spray. Similarly, in the absence of field margin vegetation, the lowest tachinid fly abundance of 3.0 was observed in plots treated with Duduthrin but was not statistically different ($P=0.05$) from plots treated with Pyeneem (3.6), Pyerin (4.0) and Nimbecidine (4.3) at 7-days post-second spray (Figure 3.9).

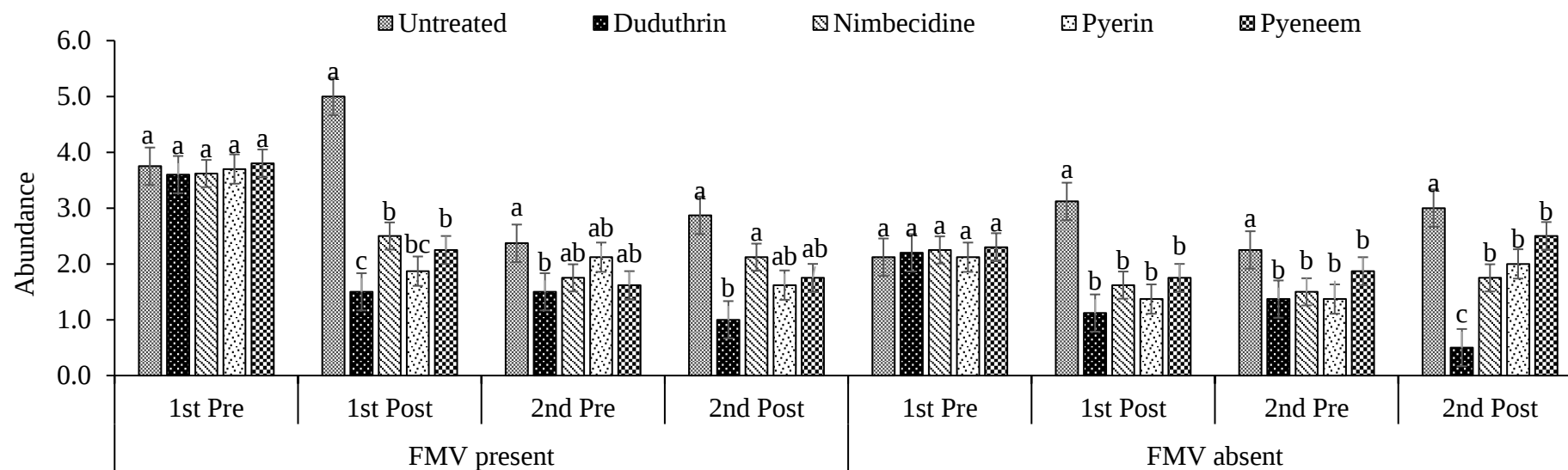


Figure 3.8: Abundance (Mean $\pm$ SE; $n=8$) of tachinid flies in dolichos bean pre-and post-botanical insecticides application in the presence or absence of field margin vegetation (May-December 2019cropping cycle). FMV = Field margin vegetation; Pre-spray 1 = spraying at 42 days after planting; Pre-spray 2= Spraying at 70 days after planting. Bars with the same letters each time before and after spraying are not significantly different using Tukey's HSD test at $P < 0.05$.

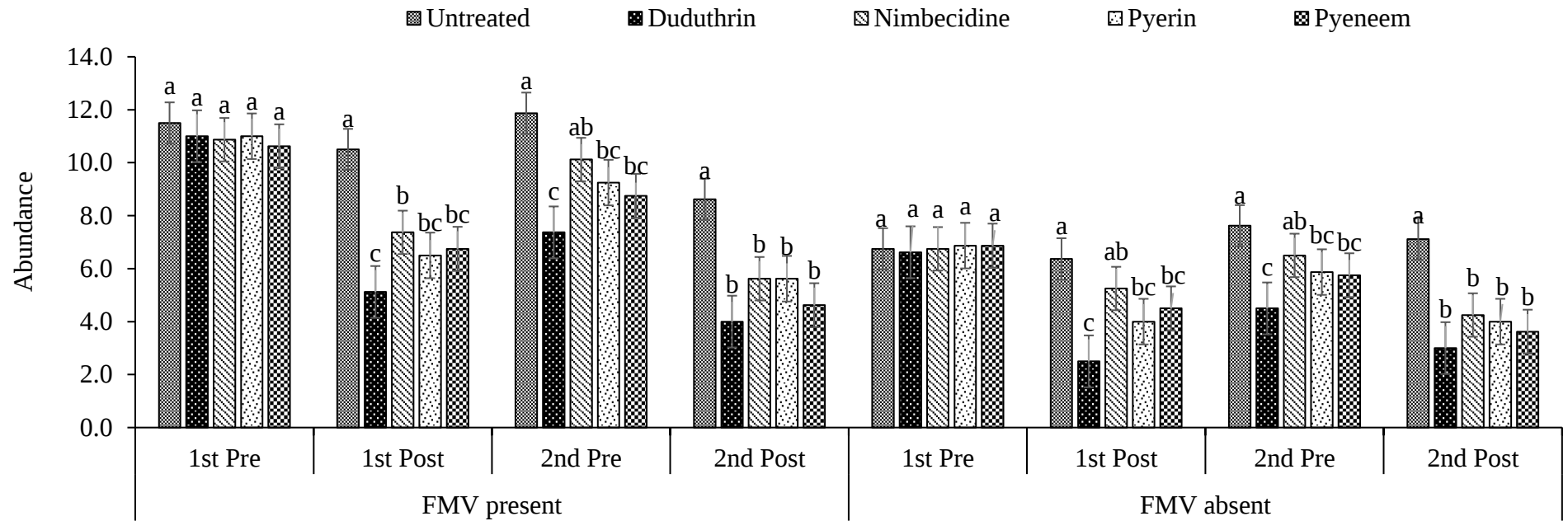


Figure 3.9: Abundance (Mean $\pm$ SE; $n=8$) of tachinid flies in dolichos bean pre-and post-botanical insecticides application in the presence or absence of field margin vegetation (March-November 2020 cropping cycle). FMV = Field margin vegetation; Pre-spray 1 = spraying at 42 days after planting; Pre-spray 2= Spraying at 70 days after planting. Bars with the same letters each time before and after spraying are not significantly different using Tukey's HSD test at $P < 0.05$.

3.4 Discussion

This study demonstrates the feasibility of combining the use of botanical insecticides and field margin vegetation to support agro-ecological intensification and sustainable management of bean aphid pests in an orphan crop legume. There was a clear interaction between botanical insecticides and field margin vegetation in this study both in suppressing bean aphids and supporting a higher abundance of natural enemies. Aphid abundance, damage severity and incidence showed a varying trend in the untreated and treated plots over the two growing cycles. During the early cycle in 2019, there was a high amount of rainfall and low temperatures which can be partially attributed to the low population build-up of bean aphids. Rainfall and temperature have been previously shown to be key abiotic factors in regulating the population density of aphids (Omkar & Pervez, 2016). Similar observations were made by Maradi *et al.* (2019) and Wosula *et al.* (2017) who reported a positive correlation in aphid population with heavy precipitation and increasing temperatures. In warm conditions, adult female aphids give birth to small nymphs which become adults in about one week and start to reproduce (Singh & Singh, 2017). There was a significant variation in aphids' infestation between the crop cycle with high aphid infestation at the vegetative dolichos growth stage. Khaemba and Ogenga-Latigo (2011) reported severe damage to black bean aphid, *Aphis fabae* Scopoli on common beans, *Phaseolus vulgaris* L. during the vegetative stage and to a lesser extent at flowering. This observation has been attributed to the fact that younger actively growing plants had more free amino acids and soluble sugars for the aphids leading to a rapid reproductive rate (Nyaanga, 2008).

In this study, low aphid abundance, damage severity and percent incidence were observed in plots that were surrounded by field margin vegetation as compared to plots that had no field margin vegetation. These findings are consistent with Karani (2017) who reported low aphid abundance, incidence and severity of damage in common bean *Phaseolus vulgaris* when intercropped with *Mesosphaerum* (syn. *Hyptis*) *suaveolens* (L.) Kuntze, *Ageratum conyzoides* L., *Tagetes minuta* L., *Ocimum suave* Willd. and *Bidens Pilosa* (L.). Mansion-Vaquié *et al.* (2020) attributed this response to chemical and physical interference which are both involved in host location and its suitability for colonization by insect pests. In addition, the inclusion of field margin vegetation around crops has been reported to harbour natural enemies that move into crop fields for biological control (Quispe *et al.*, 2017). Currently, the management of aphids and other sap-sucking pests majorly relies on broad-spectrum insecticides which are known to cause high mortality on natural enemies (Pezzini & Koch, 2015). The synthetic insecticide, Duthrin, emerged as the most effective insecticide in

reducing aphid infestation which was expected considering that it is a broad-spectrum insecticide. The effectiveness of natural pyrethrins, neem oil and azadirachtin against aphids and other sap-sucking pests has been widely documented in other crops such as common bean (*Phaseolus vulgaris*), soybean (*Glycine max* L. Merr.) and chickpea (*Cicer arietinum* L.) (Fite *et al.*, 2020; Nahashon *et al.*, 2016; Pezzini & Koch, 2015). Botanical insecticides have been reported to have variable efficacies which can be partly attributed to the difference in the mechanism of action of the insecticides and the enzymatic response of the insect species (Sisay *et al.*, 2019). Neem-based insecticides are known to be effective against aphids with repellent and antifeedant effects as well as growth and reproduction inhibition (Ulrichs *et al.*, 2001). However, botanical insecticides have been shown to have rapid degradation due to the instability of the active ingredients under ultraviolet light and ambient temperature. Low residual persistence of botanical insecticides has been considered as a disadvantage because of the need to spray botanical insecticides more frequently for effective control of insect pests (Tian *et al.*, 2021).

Conservation biological control practices through the provision of alternative habitats and food resources for natural enemies are well-known practices that can enhance ecosystem services (Amoabeng *et al.*, 2020; Mkenda *et al.*, 2015). The field margin vegetation is reported to provide shelter and floral resources for natural enemies, as well as a potential buffer against migrating pests (Bianchi & Wäckers, 2008; Knapp & Řezáč, 2015; Skirvin *et al.*, 2011). In a study by Mkenda *et al.* (2019) parasitoids were in higher abundance in the field margin plants compared to the centre of the crop. The study further demonstrated that to realize the benefits provided by field margin plants, the quality of plants as well as farm size and distance from crops to margin plants play a significant role in enhancing not only natural enemy abundance but also their activity in suppressing insect pest populations. If protected or enhanced, natural enemies can complement insecticide treatments by preying on pests that survived insecticide application or that recolonized crops after treatment, thereby reducing the likelihood of pest resurgence (Nilsson *et al.*, 2016).

Duduthrin-treated plots had the lowest natural enemy abundance which was more severe in plots not surrounded by field margin vegetation. The low abundance of natural enemies can be attributed to the high toxicity of lambda-cyhalothrin, which suppresses populations of both insect pests and their natural enemies (Mkenda *et al.*, 2015; Mkindi *et al.*, 2017). Botanical insecticides such as Nimbecidine and Pyeneem had a low influence on the abundance of natural enemies including parasitic wasps, ladybird beetles and tachinid flies in dilocho crop fields. Results indicated that the botanical insecticides were generally less

harmful to natural enemies than the synthetic insecticide (Duduthrin) as documented by other authors (Campos *et al.*, 2019; Soares *et al.*, 2019; Tembo *et al.*, 2018). However, mortality and recovery of insects after exposure to botanical insecticide have been shown to vary across insect families. Khan *et al.* (2015) reported low adult mortality of six-spotted ladybird beetles (*Menochilus sexmaculatus* Fab.) family Coccinellidae when exposed to neem oil. Similarly, lacewings (Chrysopidae) have been shown to have a high tolerance to pyrethrins due to increased levels of pyrethroid esterase (Amarasekare & Shearer, 2013). In another study, El-Wakeil *et al.* (2006) reported no mortality of lacewings due to neem-based pesticides like NSE 5%, Neemark, Achook, and Nimbecidine each at 0.003% w/v. Studies on Hymenoptera parasitoids have shown variable outcomes after exposure to botanical insecticides. High mortality on adult parasitoids, decreased parasitism and low parasitoid emergence after exposure to neem-based botanical insecticide have been demonstrated (Monsreal-Ceballos *et al.*, 2018). However, the egg parasitoid *Trichogramma pretiosum* showed low mortality when treated with azadirachtin (Almeida *et al.*, 2010). The difference in parasitoid response to botanical insecticides has been attributed to factors such as active ingredients, type of exposure, parasitoid species and stage of development (Monsreal-Ceballos *et al.*, 2018). Tian *et al.* (2021) reported a high reduction in the population of sucking pest parasitoids on a tea plantation. Similar results were observed by Jansen *et al.* (2010) who reported high mortality of beneficial arthropod species, such as the parasitic wasp *Aphidius rhopalosiphi* (Destefani-Perez) (Hymenoptera: Braconidae) and the ladybird beetles *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) after exposure to pyrethrins. To reduce high toxicological risks of botanical insecticides such as pyrethrins on natural enemies precautions should be taken to ensure that the botanical insecticides are applied at the recommended rates (Pezzini & Koch, 2015).

The results obtained in this study showed that botanical insecticides such as Pyerin and Pyneem can provide effective control of bean aphids in dolichos. Results further indicated that botanical insecticides can reduce aphids' abundance, damage severity and percent incidence to levels comparable to synthetic insecticide, Duduthrin. However, the botanical insecticides showed a higher abundance of natural enemies compared to Duduthrin. Considering that bean aphids exhibit a high reproduction rate and concerns have been raised on resistance to synthetic insecticides, the use of botanical insecticides may be a suitable alternative to synthetic insecticides due to their high efficacies against bean aphids and their compatibility with natural enemies. The use of botanical insecticides should however be integrated with other control measures such as biological control to increase the effectiveness of botanical insecticides for managing bean aphids in dolichos bean

CHAPTER FOUR

EFFECT OF BOTANICAL INSECTICIDES AND FIELD MARGIN VEGETATION ON GROWTH AND YIELD OF DOLICHOS BEAN (*Lablab purpureus* L.)

Abstract

Dolichos bean (*Lablab purpureus* L.) is an important grain legume within the smallholder farmers acting as a source of food and income. Severe insect pest infestation has been reported to cause yield losses of up to 40% in dolichos bean production. Botanical insecticides of neem oils and natural pyrethrin have been widely commercialized and are considered to be compatible with conservation biological control. However, the developed commercial botanical insecticides are rarely tested on orphan crop legumes such as dolichos bean and if applied, it is done in isolation with no provision of non-crop refuge in or around the field margins. A field study was conducted to determine the effect of selected commercial botanical insecticides and field margin vegetation on the growth and yield of dolichos bean under field conditions. Treatments (Pyerin75EC®, Nimbecidine® and Pyeneem 20EC®, Duduthrin 1.75EC® and untreated) were laid out in a randomized complete block design (RCBD) in which field plots were either surrounded by field margin vegetation (FMV) or with no FMV. Results showed that in the presence of field margin vegetation, higher (2.38-2.91 tons ha⁻¹) grain yield was observed as compared to no field margin vegetation (1.95-2.50 tons ha⁻¹) which was consistent across the 2019 and 2020 cropping cycles. Similarly in plots that were surrounded by FMV higher number of undamaged pods per plant (28.20-44.45) were observed as compared to no FMV (22.50-27.00). High grain yields of 2.55-3.04 and 2.95-3.23 tons/ha were observed for both botanical insecticides and synthetic insecticides in the presence of field margin vegetation during the 2019 and 2020 cropping cycles, respectively. These results demonstrate that combining commercial botanical insecticides and field margin vegetation holds greater potential for improving the yield of neglected legume crops, which is an important step towards food and nutrition security for smallholder farmers.

4.1 Introduction

Dolichos bean (*Lablab purpureus* L.) is an important grain legume providing food to humans, feeds for livestock, soil fertility improvement and a source of income for smallholder farmers. Despite its importance in the food and farming systems, the dolichos bean has remained neglected and is categorized as an orphan crop (Maass *et al.*, 2010). Insect pests including bean aphids (*Aphis fabae*) are one of the major limiting factors in grain legume yields

in the tropics (Abate & Ampofo, 2003). Pre- and post-harvest losses due to insect pests of legume crops are estimated to be between 30-90% with a potential total yield loss if the crops are unprotected (Cork *et al.*, 2009).

Dolichos bean is known to host a wide range of insect pests that cause significant economic loss (Forsythe, 2019). In addition, the infestation can occur at all growth stages and in grain storage (Abate & Ampofo, 2003). The cost of spraying insecticides is considered to be high as farmers have to spray their dolichos crops two to three times during the crop cycle to counter insects' populations and related damage for better yields. However, high bean aphid infestation occasioned by favourable environmental conditions has made use of synthetic insecticides costly as farmers have to undertake repeated sprays (Forsythe, 2019). The use of botanical insecticides has been established as an eco-friendly and sustainable pest control strategy (Stevenson *et al.*, 2017). The economics and cost-benefits of smallholder use of homemade crude plant extracts for pest control have proven to be favourable in many farmer situations (Mkenda *et al.*, 2015; Tembo *et al.*, 2018). However, commercialized botanical insecticides are occasioned by high market prices but their uptake and promotion can be facilitated by increasing evidence of potential benefits of low risks to human health, the environment and beneficial insects (Pavela, 2016; Sola *et al.*, 2014).

Integrated pest management (IPM) aims at combining various pest management programs to maintain the insect pest population below the economic injury level (Dedryver *et al.*, 2010; Khan *et al.*, 2015). In addition, the integration of two or more pest management programs can result in long-term pest suppression than one program in isolation. Conservation biological control and botanical insecticides have been widely reported in isolation with the main focus either on insect pest damage or beneficial insect population (Kati *et al.*, 2021; Rioba & Stevenson, 2020). To ascertain the viability of integrating two or more programs, the combined effects on insect pest damage and natural enemies populations as well as the overall crop yield is important to enhance uptake and adoption (Amoabeng *et al.*, 2020). Amoabeng *et al.* (2020) reported higher yields in cabbage (*Brassica oleracea*) when conservation biological control was integrated with botanical insecticides. However, the yield potential of orphan legume crops on the integration of conservation biological control and botanical insecticides has remained unclear. Therefore, this study was conducted to determine the effect of selected commercial botanical insecticides and field margin vegetation on the yield potential of dolichos bean under field conditions.

4.2 Materials and methods

4.2.1 Experimental site and procedure

The experimental area, climatic conditions and weather patterns during the study period are described in **section 3.2.1**. Similarly, the experimental treatments and design that were adopted for this study are described in **sections 3.2.2**.

4.2.2 Data collection

Grain yield, number of undamaged pods per plant, number of damaged pods per plant, number of seeds per pod, 100 seed weight and plant height were collected at physiological maturity when pods turned brown. Plant height was measured at vegetative growth stages and physiological maturity from ground level to the tip of the main stem using a 1m measuring wooden ruler. Above-ground biomass from each treatment was taken from 10 plants randomly selected from the middle five rows. This was a destructive sampling where the selected plants were uprooted at the late podding stage when the plants were expected to be close to the peak of dry matter accumulation. The plants were dried at 65 °C in an oven for 24 hours and dry weight was observed. The number of pods per plant was counted in each plant from 10 plants randomly selected from the inner middle rows categorized as either clean or damaged. Similarly, the number of seeds per pod was determined by threshing each pod and counting the seeds. The weight of a hundred seeds was determined using a digital QZ-161 electronic kitchen scale (Comglobal Solutions, India). For grain yield, pods were harvested separately within the sampling area for each treatment. Pods were sun-dried for two days and threshed with the moisture content observed using a draminski digital moisture meter (Dramiński S.A., Poland). After attaining 13 % moisture content, grains from each treatment were weighed separately using a portable weighing digital scale (Comglobal Solutions, India) and converted to tons ha⁻¹ using equation 4.1:

$$GY = \frac{GWP \times 10}{HA (m^2)} \dots\dots\dots (4.1)$$

Where GY=Grain yield in tons/ha, GWP= Grain weight per plot and HA= Harvest area in m²

4.2.3 Data analyses

Data on plant height, number of pods per plant, number of seeds per pod, 100 seed weight, grain yield and above-ground biomass were subjected to analysis of variance using a general linear model in SAS version 9.2. The statistical model used for the analysis is described in equation 4.2.

$$Y_{ijkl} = \mu + F_i + R_j + I_k + FI_{ik} + \mathcal{E}_{ijkl} \dots \dots \dots (4.2)$$

Where Y_{ijk} is the observation of experimental units, μ Population means, F_i effect of i^{th} field margin vegetation, R_j effect of j^{th} replicate, I_k effect of k^{th} insecticide, FI_{ik} effect of interaction between i^{th} field margin vegetation and k^{th} insecticide and $\mathcal{E}_{ijk}$ random error.

The means of treatment and interactions were separated using Tukey's Honestly Significant Difference (HSD) test at a significant level of 5% (Tukey, 1949) described in equation 3.2.

4.3 Results

4.3.1 Effect of cropping cycle and field margin vegetation on undamaged pods, damaged pods, seeds per pod, 100-seed weight, grain yield and above-ground biomass

Results showed that field margin vegetation and botanical insecticides had a significant effect on undamaged pods ($P < 0.001$), damaged pods ($P < 0.001$), grain yield ($P < 0.001$) and above-ground biomass ($P < 0.001$). Field margin vegetation was also significant for undamaged pods ($P < 0.001$), damaged pods ($P < 0.001$), grain yield ($P < 0.001$) and above-ground biomass ($P < 0.001$). The results further showed that the three-way interaction between cycle $\times$ botanical insecticides $\times$ field margin vegetation was significant for undamaged pods ($P < 0.001$), 100-seed weight ($P < 0.001$) and grain yield ($P < 0.001$) (Appendix E). Generally, in the presence of field margin vegetation, a higher (2.38-2.91 tons/ha) grain yield was observed as compared to no field margin vegetation (1.95-2.50 tons/ha) which was consistent across the two cropping cycles (Appendix F). A high number of undamaged pods, seeds per pod, 100-seed weight and above-ground biomass were observed in plots surrounded by field margin vegetation both in May-December 2019 and March-November 2020 cropping cycles (Table 4.1).

Table 4.1: Mean ($\pm$ SE) of undamaged pods/plant, damaged pod/plant, seeds/pod, 100seedweight, grain yield and biomass in margin vegetation during 2019 and 2020 cropping cycle.

May-December 2019 Cycle							
FMV	Undamaged pods/plant	Damaged pods/plant	Seeds/pod	100-Seed weight (g)	Grain yield (tons/ha)	Biomass (tons/ha)	Plant height (m)
Present	28.20 $\pm$ 0.90 ^a	15.60 $\pm$ 1.27 ^b	3.71 $\pm$ 0.03 ^a	27.28 $\pm$ 0.35 ^a	2.38 $\pm$ 0.15 ^a	8.94 $\pm$ 0.32 ^a	1.26 $\pm$ 0.06 ^a
Absent	22.55 $\pm$ 0.98 ^b	20.55 $\pm$ 1.59 ^a	3.55 $\pm$ 0.07 ^b	23.94 $\pm$ 0.54 ^b	1.95 $\pm$ 0.12 ^b	6.34 $\pm$ 0.25 ^b	0.89 $\pm$ 0.02 ^b
MSD (0.05)	0.68	0.58	0.15	0.40	0.03	0.15	0.13
March-November 2020 Cycle							
FMV	Undamaged pods/plant	Damaged pods/plant	Seeds/pod	100-Seed weight (g)	Grain yield (tons/ha)	Biomass (tons/ha)	Plant height (m)
Present	44.45 $\pm$ 1.81 ^a	19.30 $\pm$ 0.66 ^b	3.63 $\pm$ 0.04 ^a	26.34 $\pm$ 0.27 ^a	2.91 $\pm$ 0.11 ^a	8.28 $\pm$ 0.35 ^a	1.01 $\pm$ 0.01 ^a
Absent	27.00 $\pm$ 1.14 ^b	25.20 $\pm$ 1.27 ^a	3.38 $\pm$ 0.04 ^b	24.87 $\pm$ 0.27 ^b	2.50 $\pm$ 0.10 ^b	5.77 $\pm$ 0.27 ^b	0.81 $\pm$ 0.02 ^b
MSD (0.05)	0.84	0.55	0.07	0.60	0.04	0.16	0.04

Means in a column followed by the same letters are not significantly different at $P < 0.05$ using Tukey's HSD test. FMV= Field margin vegetation; MSD = Minimum significant difference.

4.3.2 Effect of botanical insecticides on undamaged pods, damaged pods, seeds per pod, 100-seed weight, grain yield and above-ground biomass

The results showed that the botanical insecticides significantly influenced the yield and yield components of the dolichos bean across the two cropping cycles. Generally, during the March-November 2020 cropping cycle, a higher number of undamaged pods per plant were observed (av. 35.73 ± 1.75) as compared to the 2019 cycle (av. 25.38 ± 0.79). During the May-December 2019 cycle, the untreated control had the lowest grain output in both the presence (1.29 ± 0.01 tons ha⁻¹) and absence (0.99 ± 0.03 tons ha⁻¹) of field margin vegetation. In the presence of field margin vegetation, the highest grain production was obtained when dolichos was treated with the botanical insecticide, Pyeneem (3.05 tons ha⁻¹) and the synthetic insecticide, Duduthrin (3.04 tons ha⁻¹). Pyerin (2.55 tons ha⁻¹) was the next best botanical insecticide for grain yield, followed by Nimbecidine (1.96 tons ha⁻¹) with field margin vegetation present. However, in the absence of field margin vegetation, grain yields were significantly lower, with Duduthrin having the highest yield (2.55 tons ha⁻¹). Pyeneem had the best grain yield (2.35 tons ha⁻¹) among botanical insecticides, followed by Pyerin (2.01 tons ha⁻¹) and Nimbecidine (1.86 tons ha⁻¹) (Table 4.2).

During the March-November 2020 cropping cycle, untreated plots had the lowest grain yield in both the presence (1.99 ± 0.01 tons ha⁻¹) and absence (1.73 ± 0.05 tons ha⁻¹) of field margin vegetation. Duduthrin-treated plots had the highest grain production of 3.24 tons ha⁻¹ in the presence of field margin vegetation, which was not significantly different from Pyeneem (3.14 tons ha⁻¹) and Pyerin (3.22 tons ha⁻¹). In the absence of field margin vegetation, Duduthrin treated plots had the highest grain production (2.85 tons ha⁻¹), which was not significantly different from Pyeneem (2.79 tons ha⁻¹) and Nimbecidine (2.71 tons ha⁻¹) treatments (Table 4.3).

Table 4. 2: Mean ($\pm$ SE) of undamaged pods/plant, damaged pod/plant, seeds/pod, 100seedweight, grain yield, biomass and plant height as affected by selected botanical insecticides and field margin vegetation during May_ December 2019 cropping cycle.

Botanicals	With Field margin vegetation						
	Undamaged pods/plant	Damaged pods/plant	Seeds/pod	100-Seed weight (g)	Grain yield (tons/ha)	Biomass (tons/ha)	Plant height (m)
Untreated	23.00 $\pm$ 1.08 ^c	25.00 $\pm$ 0.41 ^a	3.58 $\pm$ 0.09 ^b	24.92 $\pm$ 0.66 ^c	1.29 $\pm$ 0.03 ^d	7.24 $\pm$ 0.22 ^d	0.81 $\pm$ 0.01 ^b
Dudutrhrin	34.50 $\pm$ 0.64 ^a	10.75 $\pm$ 0.48 ^d	3.75 $\pm$ 0.03 ^{ab}	28.99 $\pm$ 0.05 ^a	3.04 $\pm$ 0.03 ^a	11.05 $\pm$ 0.06 ^a	1.44 $\pm$ 0.21 ^a
Nimbecidine	26.50 $\pm$ 0.64 ^b	18.50 $\pm$ 0.65 ^b	3.68 $\pm$ 0.08 ^{ab}	26.60 $\pm$ 0.26 ^b	1.96 $\pm$ 0.02 ^c	8.84 $\pm$ 0.07 ^c	1.56 $\pm$ 0.04 ^a
Pyerin	28.25 $\pm$ 0.47 ^b	12.50 $\pm$ 0.65 ^c	3.68 $\pm$ 0.09 ^{ab}	27.98 $\pm$ 0.05 ^{ab}	2.55 $\pm$ 0.03 ^b	7.76 $\pm$ 0.16 ^d	1.01 $\pm$ 0.01 ^{ab}
Pyeneem	28.75 $\pm$ 0.62 ^b	11.25 $\pm$ 0.25 ^{cd}	3.85 $\pm$ 0.03 ^a	27.92 $\pm$ 0.12 ^{ab}	3.05 $\pm$ 0.03 ^a	9.84 $\pm$ 0.09 ^b	1.54 $\pm$ 0.24 ^a
MSD (0.05)	2.93	1.63	0.26	1.46	0.14	0.64	0.60
Botanicals	Without field margin vegetation						
	Undamaged pods/plant	Damaged pods/plant	Seeds/pod	100-Seed weight (g)	Grain yield (tons/ha)	Biomass (tons/ha)	Plant height (m)
Untreated	15.75 $\pm$ 0.47 ^c	33.50 $\pm$ 0.65 ^a	3.38 $\pm$ 0.20 ^a	20.20 $\pm$ 0.36 ^d	0.99 $\pm$ 0.01 ^e	5.45 $\pm$ 0.12 ^c	0.67 $\pm$ 0.00 ^c
Dudutrhrin	26.00 $\pm$ 0.40 ^a	15.50 $\pm$ 0.29 ^c	3.45 $\pm$ 0.22 ^a	26.51 $\pm$ 0.30 ^a	2.55 $\pm$ 0.04 ^a	8.24 $\pm$ 0.11 ^a	0.97 $\pm$ 0.03 ^a
Nimbecidine	19.50 $\pm$ 0.64 ^b	21.75 $\pm$ 0.48 ^b	3.55 $\pm$ 0.13 ^a	24.96 $\pm$ 0.06 ^b	1.86 $\pm$ 0.02 ^d	6.47 $\pm$ 0.10 ^b	0.98 $\pm$ 0.04 ^a
Pyerin	26.00 $\pm$ 0.40 ^a	16.00 $\pm$ 0.41 ^c	3.70 $\pm$ 0.07 ^a	22.54 $\pm$ 0.31 ^c	2.01 $\pm$ 0.03 ^c	5.10 $\pm$ 0.03 ^c	0.82 $\pm$ 0.02 ^b
Pyeneem	25.50 $\pm$ 0.29 ^a	16.00 $\pm$ 0.41 ^c	3.65 $\pm$ 0.12 ^a	25.50 $\pm$ 0.36 ^{ab}	2.35 $\pm$ 0.03 ^b	6.45 $\pm$ 0.12 ^b	1.02 $\pm$ 0.01 ^a
MSD (0.05)	1.86	2.23	0.74	1.29	0.13	0.45	0.12

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

MSD= Minimum significant difference

Table 4.3: Mean ($\pm$ SE) of undamaged pods/plant, damaged pod/plant, seeds/pod, 100seedweight, grain yield, biomass and plant height as affected by selected botanical insecticides and field margin vegetation during March_ November 2020 cropping cycle.

Botanicals	With Field margin vegetation						
	Undamaged pods/plant	Damaged pods/plant	Seeds/pod	100-Seed weight (g)	Grain yield (tons/ha)	Biomass (tons/ha)	Plant height (m)
Untreated	30.50 $\pm$ 0.29 ^d	24.25 $\pm$ 0.48 ^a	3.45 $\pm$ 0.03 ^b	25.73 $\pm$ 0.69 ^b	1.99 $\pm$ 0.01 ^c	5.49 $\pm$ 0.06 ^d	0.88 $\pm$ 0.03 ^c
Dudutrhrin	53.75 $\pm$ 0.63 ^a	16.5 $\pm$ 0.29 ^c	3.75 $\pm$ 0.03 ^a	28.20 $\pm$ 0.20 ^a	3.24 $\pm$ 0.04 ^a	9.44 $\pm$ 0.23 ^{ab}	1.03 $\pm$ 0.01 ^{ab}
Nimbecidine	44.00 $\pm$ 0.58 ^c	20.50 $\pm$ 0.29 ^b	3.55 $\pm$ 0.12 ^{ab}	25.22 $\pm$ 0.13 ^b	2.95 $\pm$ 0.02 ^b	8.07 $\pm$ 0.03 ^c	1.00 $\pm$ 0.03 ^b
Pyerin	44.50 $\pm$ 0.65 ^c	17.50 $\pm$ 0.29 ^c	3.68 $\pm$ 0.06 ^{ab}	26.05 $\pm$ 0.15 ^b	3.22 $\pm$ 0.03 ^a	8.70 $\pm$ 0.22 ^{bc}	1.10 $\pm$ 0.01 ^a
Pyneem	49.50 $\pm$ 0.65 ^b	17.75 $\pm$ 0.48 ^c	3.70 $\pm$ 0.04 ^{ab}	26.53 $\pm$ 0.22 ^b	3.14 $\pm$ 0.03 ^a	9.71 $\pm$ 0.16 ^a	1.04 $\pm$ 0.01 ^{ab}
MSD (0.05)	2.56	1.58	0.27	1.55	0.14	0.79	0.09
Botanicals	Without Field margin vegetation						
	Undamaged pods/plant	Damaged pods/plant	Seeds/pod	100-Seed weight (g)	Grain yield (tons/ha)	Biomass (tons/ha)	Plant height (m)
Untreated	19.00 $\pm$ 0.41 ^c	35.75 $\pm$ 0.48 ^a	3.18 $\pm$ 0.06 ^c	25.10 $\pm$ 0.95 ^a	1.73 $\pm$ 0.05 ^c	3.47 $\pm$ 0.03 ^d	0.55 $\pm$ 0.02 ^c
Dudutrhrin	32.25 $\pm$ 1.03 ^a	20.75 $\pm$ 0.25 ^d	3.35 $\pm$ 0.06 ^{bc}	25.61 $\pm$ 0.33 ^a	2.85 $\pm$ 0.03 ^a	6.66 $\pm$ 0.08 ^a	0.86 $\pm$ 0.01 ^b
Nimbecidine	29.75 $\pm$ 0.85 ^a	25.25 $\pm$ 0.25 ^b	3.65 $\pm$ 0.06 ^a	23.76 $\pm$ 0.23 ^a	2.71 $\pm$ 0.01 ^a	6.07 $\pm$ 0.06 ^c	0.83 $\pm$ 0.03 ^b
Pyerin	24.25 $\pm$ 0.48 ^b	22.75 $\pm$ 0.48 ^c	3.25 $\pm$ 0.03 ^{bc}	25.12 $\pm$ 0.38 ^a	2.41 $\pm$ 0.02 ^b	6.24 $\pm$ 0.03 ^{bc}	0.87 $\pm$ 0.02 ^{ab}
Pyneem	29.75 $\pm$ 0.85 ^a	21.50 $\pm$ 0.65 ^{cd}	3.48 $\pm$ 0.06 ^{ab}	24.77 $\pm$ 0.63 ^a	2.79 $\pm$ 0.03 ^a	6.41 $\pm$ 0.09 ^{ab}	0.94 $\pm$ 0.01 ^a
MSD (0.05)	3.47	1.84	0.27	2.16	0.13	0.28	0.07

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

MSD = Minimum significant difference.

4.4 Discussion

The results from the study indicated that providing non-crop habitat around the field margins could increase the grain yield of the dolichos bean. Results showed that the use of botanical insecticides on dolichos bean could lead to grain yield similar to synthetic insecticides. However, the integration of field margin vegetation and botanical insecticides led to higher grain yield compared to the use of botanical insecticides without the field margin vegetation. Untreated plots had the lowest grain yield both in the presence and absence of field margin vegetation. The low yield can be attributed to higher levels of aphid infestation observed in the untreated plots. Bean aphids were observed across all growth stages in untreated plots with higher aphid abundance during the early and late vegetative growth stages. Dolichos bean is known to have slow growth during the vegetative stage and aphid infestation at this stage makes it highly susceptible to stunted growth. Aphids cause leaves to curl reducing the photosynthetic ability of the crop. Therefore, reduced plant height and grain yield observed in the untreated plots can be attributed to the high bean aphid infestation level observed during the critical growth stages of the dolichos bean. These findings are consistent with Kayange *et al.* (2019) who reported reduced grain yield in common bean (*Phaseolus vulgaris*) due to bean aphids infestation.

The botanical insecticides and synthetic insecticides (Duduthrin) resulted in a higher grain yield than the untreated control. Concerning aphid infestation levels, Nimbecidine was generally the least effective botanical insecticide for reducing aphid infestations. This is further reflected in bean yields obtained where Nimbecidine had the lowest yields of the three botanical insecticides. Considering that the impact of the three botanical insecticides on natural enemy populations was generally comparable, the lower yield achieved with Nimbecidine will reduce farmer profitability in comparison to the use of Pyerin or Pyeneem with no clear benefit of using Nimbecidine to protect crops. Although Pyerin and Pyeneem botanical insecticides were generally as effective in reducing aphid infestations as the synthetic Duduthrin, the botanical insecticide-treated plots showed a higher abundance of natural enemies post-spray compared to the synthetic.

Bean aphids have been shown to have a significant effect on the numbers of pods per plant, numbers of seeds per pod, the 100-seed weight (g), above-ground biomass (tons/ha) and grain yield (tons/ha). In the untreated plots (negative control), low numbers of pods per plant, numbers of seeds per pod, 100-seed weight, above-ground biomass and grain yield were observed which directly related to high bean aphid infestation. These findings are in agreement with Mwangi *et al.* (2008) who reported significant grain yield reduction on susceptible

common bean (*Phaseolus vulgaris*) varieties to *Aphis fabae*. In plots treated with botanical insecticides and synthetic insecticide, Duduthrin, a higher number of undamaged pods per plant were observed as compared to untreated control both in the presence and absence of field margin vegetation. Results further showed that the use of botanical insecticides can result in similar yields of dolichos bean to those achieved with synthetic insecticides which were enhanced by the inclusion of field margin plants around cropping areas. In related studies, final grain yield has been reported to be positively correlated with the number of pods per plant, seeds per pod and 100- seed weight (Karani, 2017; Tembo *et al.*, 2018). Therefore, increased grain yield in the insecticide-treated plots can partly be attributed to higher pods per plant, seeds per pod and heavier seeds as manifested in 100-seed weight.

Integration of field margin vegetation improved the effectiveness of botanical insecticides in both suppressing the bean aphid population and increasing the abundance of natural enemies. The provision of field margin vegetation further enhanced the suppression of bean aphids and a higher abundance of natural enemies which are known to prey on bean aphids. Furthermore, botanical extracts have been documented to improve plant nutrition as a green fertilizer hence provision of additional plant nutrients. In a study conducted by Mkindi *et al.* (2020) insecticides of plant origin were shown to have direct effects on inducing systemic plant responses improving plant vigour by functioning as a green fertilizer. Since natural pyrethrins and neem are of plant origin, higher grain yield and above-ground biomass in plots treated with these insecticides can be partly attributed to the addition of nitrogen from the botanicals. However, there is a need to ascertain this fact by testing these botanical insecticides alongside commercial foliar fertilizer under pest-free conditions. In this study, it was observed that the integration of field margin vegetation and botanical insecticides can result in the effective management of bean aphids on orphan crop legumes with corresponding higher yields. The combination of botanical insecticides and field margin vegetation exhibited higher grain yield and above-ground biomass thus having great potential to increase dolichos production and incomes of smallholder farmers.

CHAPTER FIVE

EFFECT OF BOTANICAL INSECTICIDES AND FIELD MARGIN VEGETATION ON RECOVERY OF LADYBIRD BEETLES (*Chelomenes lunata*, COLEOPTERA: COCCINELLIDAE) IN DOLICHOS BEAN

Abstract

Conservation biological control involving ladybird beetles includes the use of selective botanical insecticides and the provision of non-crop habitat. This study was conducted to evaluate the recovery of ladybird beetles after the application of botanical insecticides (Pyrin75EC[®], Nimbecidine[®] and Pyeneem 20EC[®]) under a restricted movement of the predators in and out of cages. Treatments were laid out in a randomized complete block design (RCBD) in field exclusion cages either with field margin vegetation (FMV) or with no FMV. The field margin plants were comprised of *Bidens Pilosa*, *Tagetes minuta*, *Ageratum conyzoides* and *Galinsoga parviflora*. The botanical insecticides and positive control (Duduthrin) were applied at the second and sixth trifoliate dolichos growth stages. The change in the numbers of ladybird beetle was converted to percent population reduction and a change in percent population density. Results revealed that the botanical insecticides had a higher number of ladybird beetle in the presence of field margin vegetation (Pyrin (4.28), Nimbecidine (3.91) and Pyeneem (4.43) as compared to no FMV (Pyrin (3.46), Nimbecidine (3.15) and Pyeneem (3.65). As compared to insecticides treated cages, untreated cages showed an increase in the number of ladybird beetles. In the presence of field margin vegetation, untreated cages had a high number of ladybird beetles (6.41) compared to no FMV (4.31). Field margin vegetation and botanical insecticides have the potential for the conservation of the ladybird beetle which is the main predator of black bean aphid in natural pest regulation.

5.1 Introduction

Biological control is one of the most effective means of achieving insect pest control and is often recommended as the first line of defense in an integrated pest management (IPM) program (Munyuli *et al.*, 2007). The family Coccinellidae, order Coleoptera consists of exclusively predaceous species (Nyaanga, 2008; Omkar & Pervez, 2016). Ladybird beetles are the most important group of insects in the family Coccinellidae which feed on a wide range of soft-bodied insect pests including aphids, whiteflies, mealybugs and scales contributing to their natural and biological control (Khan *et al.*, 2015; Schiess *et al.*, 2017). The aphidophagous

ladybird beetle adults and larvae largely rely on aphids as part of their diets, although they may occasionally use alternative floral resources such as pollen and nectar at low aphid density (Pan *et al.*, 2020).

Insecticides have been considered a key component of insect pest suppression in IPM with little regard to minimizing the disruption of natural enemies and other beneficial organisms (Campos *et al.*, 2019; Rioba & Stevenson, 2020). The indiscriminate and excessive use of pesticides has been reported to destroy beneficial organisms such as predators, parasitoids and pollinators (Paredes *et al.*, 2013). Much attention has been directed to horticulture where vegetable and flower growers use fewer insecticides or no insecticides due to concern about maximum residues levels by the export market (EFSA, 2016). However, the use of conventional insecticides also affects the natural enemy populations, including aphidophagous ladybird beetles, although with variable results (Khan *et al.*, 2015; Schiess *et al.*, 2017).

Studies conducted so far indicate that neem and pyrethrin are comparatively safe from natural enemies (Almeida *et al.*, 2010; Pezzini & Koch, 2015). The available evidence shows that botanical insecticides are biodegradable in contrast to persistent synthetic insecticides (Sola *et al.*, 2014). The coccinellid beetles are considered to be of great economic importance in natural pest regulation (Sloggett, 2021). However, coccinellids can be exposed to insecticides and contaminated during pesticide applications by direct contact with pesticide residues and by consuming prey exposed to insecticides (Depalo *et al.*, 2017; Soares *et al.*, 2019). The International Organization for Biological Control (IOBC) has developed standard methods for evaluating the side effects of pesticides with subsequent four toxicity categories depending on the degree of mortality they cause to beneficial organisms (El-Wakil *et al.*, 2014). With the limited information on the overall recovery of natural enemies after insecticide application, a study was conducted to determine the effect of botanical insecticides and field margin vegetation on the recovery of ladybird beetles, the major aphid predators.

5.2 Materials and methods

5.2.1 Rearing of black bean aphids and predators (Ladybird beetles-*Chelomenes lunata*)

Dolichos variety DL-1002 was used as a host plant to raise black bean aphids (*Aphis fabae*) in cages under a moveable rain shelter. The rearing of both aphid colonies and ladybird beetles was carried out according to Nyaanga (2008). The dolichos were planted in 5-litre capacity planting plastic pots. Each pot was sown with two seeds which were thinned to one 21 days after planting. In establishing aphid colonies in the laboratory alate (winged) aphids

were used due to their characteristic fast reproduction (Mkindi *et al.*, 2017). Watering was done at the base of the crops to prevent dislodging of established aphid colonies. Dolichos plants with well-established colonies were selected and used to rear natural enemies in separate cages under the rain shelter. Aphidophagous ladybird beetles (*Chelomenes lunata*) were collected from unsprayed fields and transferred to cages containing aphid-infested dolichos plants. Fresh supplies of aphid-infested dolichos plants were introduced in the ladybird beetles rearing cages upon colony depletion to ensure a continuous supply of prey for the beetles. From the rearing cages, both aphid colonies and newly emerged adult ladybird beetles were used as the source of infestation.

5.2.2 Experimental procedure

A field cage exclusion experiment was conducted to test the role of field margin plant species on the recovery of ladybird beetles from the application of botanical insecticides compared to Duduthrin (positive control). This was a 2-factor experiment consisting of 3 commercial botanical insecticides (Nimbecidine, Pyerin and Pyeneem; positive (Duduthrin) and negative (untreated) controls and field margin vegetation at two levels (present or absent). The field margin plant species consisted of *Bidens pilosa*, *Tagetes minuta*, *Ageratum conyzoides* and *Galinsoga parviflora*. The seeds of the four plant species were mixed in equal proportions and germinated in a plastic pot which was placed in treatments having field margin plant species. Each treatment combination was placed in a 1.5m high by 1m length by 1m width exclusion cage consisting of a round bar steel (diameter 5.8mm, R-6) frame covered with polyester netting (mesh size 1 × 0.3mm, thread thickness 0.1mm) (Plate 6.1). The polyester nets were extended 0.2m below the soil surface to prevent the entry of other insects. Access in and out of cages was provided by a vent held closely by white zip in each cage unit. Planting pots 5-litre capacity were used to plant dolichos and field margin plant species. Dolichos variety DL-1002 was planted 2 seeds per pot and thinned to one seedling per pot 21 days after planting based on the vigour.

Potted plants were maintained through adequate watering after every two days to protect the plants from moisture stress. The watering was done at the base of the crops to prevent dislodging of inoculated aphid colonies. Before insecticide application, aphids and ladybird beetle were introduced onto the plants. Using camel brushes 20 aphids were used to infest each plant 7 days pre-spraying. Newly emerged five ladybird beetles were introduced to each cage 3 days pre-spraying. The insects were left to settle on dolichos plants for up to three days before insecticide application. The spraying regimes were similar to field experiments

maintaining the growth stages and insecticides application rate as described under section 3.2.2. Insecticide applications were done three days after the introduction of aphids and ladybird beetles using a hand sprayer.



Figure 5.1: Cage experiment layout: sample cage experimental layout (A), cage without field margin plants (B) and cage with field margin plants (C).

5.2.3 Data collection

Before insecticide application, the number of live ladybird beetles on plants in each pot was counted and the dolichos plants assigned to each treatment were sprayed with the corresponding insecticide. Plants in untreated control were not sprayed throughout the growing period. The ladybird beetle populations for the second spraying were based on the build-up after the first introduction 42 days after planting. Pre-treatment counts were taken one day before spraying while the post-treatment counts were undertaken after 7, 14 and 21 days after spraying. The population of ladybird beetles was categorized into two as a change in percent population density and effectiveness measured in terms of percent reduction relative to the untreated control.

The change in population density was calculated according to Saleem *et al.* (2019) shown in equation 5.1 below:

$$\text{CPD (\%)} = \left(\frac{X_i - X_0}{X_0} \right) \times 100 \dots \dots \dots (5.1)$$

Where CPD is a change in population density, X_0 is the mean number of live insects before treatment, X_i is the mean number of live insects at the assessment after treatment. Positive values in the table indicate an increase in the insect population.

The percent reduction in population was calculated according to Henderson-Tilton's formula (Henderson & Tilton, 1955) shown in equation 5.2 below:

$$RP (\%) = 1 - \left(\frac{T_a \times C_b}{T_b \times C_a} \right) \times 100 \dots\dots\dots (5.2)$$

where: RP= reduction in population Ta = number of insects after treatment, Tb = number of insects before treatment, Ca = number of insects in control plots after treatment, Cb = number of insects in control plots before treatment.

5.2.4 Data analyses

Data on percent change in population density and percent reduction were first subjected to arcsine transformation to correct for heterogeneity of treatment variances. analysis through SAS version 9.2 (SAS, 2011) Effects of botanical insecticides, field margin vegetation and their interactions were subjected to analysis of Variance (ANOVA) for change in population density, percent and number of ladybird beetles using SAS version 9.2 (SAS, 2011) as described in equation 5.3. The treatment means were separated using Tukey's Honestly Significant Difference (HSD) test at a 5% significance level (Tukey, 1949).

$$Y_{ijkl} = \mu + F_i + R_j + I_k + EI_{ik} + \mathcal{E}_{ijkl} \dots\dots\dots (5.3)$$

Where Y_{ijk} is the observation of experimental units, μ Population means, F_i effect of i^{th} field margin vegetation, R_j effect of j^{th} replicate, I_k effect of k^{th} insecticide, EI_{ik} effect of interaction between i^{th} field margin vegetation and k^{th} insecticide and $\mathcal{E}_{ijk}$ random error.

5.3 Results

5.3.1 Analysis of variance for number, percent change of population density and percent reduction of ladybird beetles

Results showed that field margin vegetation significantly influenced the numbers of ladybird beetles ($P < 0.0001$) and percent reduction ($P < 0.0001$) and change in percent population density ($P < 0.0001$). The two-way interaction between botanical insecticide and margin vegetation was significant for the number of ladybird beetles ($P < 0.0001$), percent reduction ($P < 0.0001$) and change in percent population density ($P < 0.0001$) (Appendix H). The results showed that the botanical insecticides had a higher number of ladybird beetles in presence of field margin vegetation Pyerin (4.28), Nimbecidine (3.91) and Pyeneem (4.43) as compared to no field margin vegetation Pyerin (3.46), Nimbecidine (3.15) and Pyeneem (3.65). As compared to insecticides treated cages, untreated cages showed a significant increasing trend in the number of ladybird beetle with a high increase observed in cages with field margin vegetation. In the presence of field margin vegetation, untreated cages had a higher number of ladybird beetles (6.41) compared to no field margin vegetation (4.31) (Appendix I).

5.3.2 Efficiency of the selected botanical insecticides and field margin vegetation on the recovery of ladybird beetles

Results showed that botanical insecticide and field margin vegetation treatments significantly affected the number of ladybird beetles ($P < 0.0001$) and the percent reduction in ladybird beetles ($P < 0.0001$). In the presence of margin vegetation, a higher number of ladybird beetles (av. 4.48) was observed as compared to no field margin vegetation (av. 3.40). In the presence of field margin vegetation, 7 days post first spraying, Duduthrin-treated cages had the highest reduction in the number of ladybird beetles (35 %) and a CPD of 35 %. The botanical insecticides at 7 days post first treatment were not different for percent reduction and percent CPD each at 20%. As compared to treated cages at 21 DAS for the first spraying, untreated control observed no change in the number of ladybird beetles (5). During the second spraying, the number of ladybird beetles started to recover. The highest recovery was observed at 21 DAS for second spraying with a lower % reduction in cages treated with botanical insecticides Pyeneem (18%) and Pyerin (19%) as compared to the positive control, Duduthrin (22%). However, at 21 DAS for second spraying untreated control observed an increase in the number of ladybird beetles (8) and CPD (13%) (Table 5.1).

Table 5.1: Population densities (Mean±SE) of ladybird beetles on dolichos bean as influenced by botanical insecticides in the presence of field margin vegetation.

With field margin vegetation				
Botanicals	First spraying			
	Pre-spray 1	7DAS		
	Ladybird No.	Ladybird No.	CPD (%)	Reduction (%)
Duduthrin	5 ^a	3.3±0.1 ^c	-35.0±5.0 ^c	35.0±2.5 ^a
Nimbecidine	5 ^a	4.0±0.0 ^b	-20.0±0.0 ^b	20.0±0.0 ^b
Pyeneem	5 ^a	4.0±0.0 ^b	-20.0±0.0 ^b	20.0±0.0 ^b
Pyerin	5 ^a	4.0±0.0 ^b	-20.0±0.0 ^b	20.0±0.0 ^b
Untreated	5 ^a	5.0±0.0 ^a	0.0±0.0 ^a	-
MSD (0.05)	0.00	0.50	10.08	10.08
14DAS				
Botanicals		Ladybird No.	CPD (%)	Reduction (%)
Duduthrin	----	3.0±0.0 ^c	-40.0±0.0 ^c	40.0±0.0 ^a
Nimbecidine	----	4.0±0.0 ^b	-20.0±0.0 ^b	20.0±0.0 ^b
Pyeneem	----	3.6±0.1 ^b	-25.0±5.0 ^b	25.0±2.5 ^b
Pyerin	----	3.6±0.1 ^b	-25.0±5.0 ^b	25.0±2.5 ^b
Untreated	----	5.0±0.0 ^a	0.0±0.0 ^a	-
MSD (0.05)	----	0.62	12.35	12.35
21DAS				
Botanicals		Ladybird No.	CPD (%)	Reduction (%)
Duduthrin	----	2.5±0.1 ^c	-50.0±5.8 ^b	50.0±2.9 ^a
Nimbecidine	----	3.5±0.1 ^b	-30.0±5.8 ^b	30.0±2.9 ^b
Pyeneem	----	3.0±0.0 ^{bc}	-40.0±0.0 ^{bc}	40.0±0.0 ^{ab}
Pyerin	----	3.0±0.0 ^{bc}	-40.0±0.0 ^{bc}	40.0±0.0 ^{ab}
Untreated	----	5.0±0.0 ^a	0.0±0.0 ^a	-
MSD (0.05)	----	0.82	16.46	16.46

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

DAS – days after spraying; Pre-spray 1 = spraying at 42 days after planting; Pre-spray 2= Spraying at 70 days after planting; MSD= Minimum significant difference; CPD (%) = change in population density; Reduction (%) = percent reduction in the number ladybird beetles.

Table 5.1 continued...

Botanicals	With field margin vegetation			
	Second spraying			
	Pre-spray 2	7DAS		
	Ladybird No.	Ladybird No.	CPD (%)	Reduction (%)
Duduthrin	4.0±0.0 ^c	2.5±0.1 ^c	-37.5±7.2 ^b	39.1±4.11 ^a
Nimbecidine	6.3±0.2 ^{ab}	3.5±0.1 ^{bc}	-42.5±8.0 ^b	45.0±2.89 ^a
Pyeneem	5.5±0.3 ^b	3.8±0.1 ^b	-31.3±2.1 ^b	33.6±0.14 ^a
Pyerin	5.0±0.3 ^{bc}	2.5±0.1 ^c	-50.0±0.0 ^b	51.6±0.79 ^a
Untreated	7.5±0.1 ^a	7.8±0.1 ^a	3.6±3.6 ^a	-
MSD (0.05)	1.45	1.18	22.84	21.11
14DAS				
Botanicals		Ladybird No.	CPD (%)	Reduction (%)
Duduthrin	----	3.0±0.0 ^c	-25.0±0.0 ^b	24.7±2.1 ^a
Nimbecidine	----	4.3±0.1 ^b	-31.2±4.8 ^b	31.1±2.5 ^a
Pyeneem	----	4.3±0.1 ^b	-20.8±8.0 ^b	21.6±2.3 ^a
Pyerin	----	3.5±0.1 ^{bc}	-27.1±10.4 ^b	27.8±3.9 ^a
Untreated	----	7.5±0.1 ^a	0.3±5.5 ^a	-
MSD (0.05)	----	1.11	20.59	19.58
21DAS				
Botanicals		Ladybird No.	CPD (%)	Reduction (%)
Duduthrin	----	3.5±0.1 ^c	-12.5±7.2 ^{ab}	22.5±3.9 ^{ab}
Nimbecidine	----	5.0±0.2 ^b	-17.0±13.5 ^b	27.8±4.4 ^a
Pyeneem	----	5.0±0.0 ^b	-6.3±10.4 ^{ab}	18.1±2.8 ^{ab}
Pyerin	----	4.5±0.1 ^{bc}	-8.3±4.8 ^{ab}	19.3±1.7 ^{ab}
Untreated	----	8.5±0.1 ^a	13.8±5.8 ^a	-
MSD (0.05)	----	1.30	27.16	23.77

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

DAS – days after spraying; Pre-spray 1 = spraying at 42 days after planting; Pre-spray 2 = Spraying at 70 days after planting; MSD= Minimum significant difference; CPD (%) = change in population density; Reduction (%) = percent reduction in the number ladybird beetles.

In the absence of field margin vegetation, a lower number of ladybird beetles was recovered post first and second sprayings. At 7 days post first spraying, Duduthrin-treated cages had the highest reduction in the number of ladybird beetles (55 %) and a CPD of 55 %. Among the botanical insecticides, at 7 days post first spraying, Nimbecidine had the least reduction in the number of ladybird beetles (15%) compared to Pyeneem (25%) and Pyerin (35%). As compared to treated cages at 21 DAS for the first spraying, a 20% reduction was observed in untreated control for the ladybird beetles which was significantly different from treated cages except for Nimbecidine (30%). During the second spraying, there was a lower reduction in the number of ladybird beetles at 21 DAS. However, Duduthrin-treated cages had the highest reduction (43%) compared to the three botanical insecticides (5%) at 21 DAS for second spraying. At 21 DAS for second spraying, untreated control observed an increase in the number of ladybird beetles (av. 4.3) and a CPD of 6% (Table 5.2).

Table 5.2: Population densities (Mean±SE) of ladybird beetles on dolichos bean as influenced by botanical insecticides in the absence of field margin vegetation.

Without field margin vegetation				
Botanicals	First spraying			
	Pre-spray 1	7DAS		
	Ladybird No.	Ladybird No.	CPD (%)	Reduction (%)
Duduthrin	5 ^a	2.3±0.1 ^d	-55.0±5.0 ^d	55.0±2.5 ^a
Nimbecidine	5 ^a	4.3±0.1 ^{ab}	-15.0±5.0 ^{ab}	15.0±2.5 ^c
Pyeneem	5 ^a	3.8±0.13 ^{bc}	-25.0±5.0 ^{bc}	25.0±2.5 ^{bc}
Pyerin	5 ^a	3.3±0.13 ^c	-35.0±5.0 ^c	35.0±2.5 ^b
Untreated	5 ^a	5.0±0.0 ^a	0.0±0.0 ^a	-
MSD (0.05)	0.00	0.92	18.40	18.40
14DAS				
Botanicals		Ladybird No.	CPD (%)	Reduction (%)
Duduthrin	----	2.0±0.0 ^c	-60.0±0.0 ^c	52.5±1.3 ^a
Nimbecidine	----	3.8±0.1 ^{ab}	-25.0±5.0 ^{ab}	11.3±3.3 ^{bc}
Pyeneem	----	3.0±0.2 ^b	-40.0±8.2 ^b	28.8±5.4 ^b
Pyerin	----	3.0±0.0 ^b	-40.0±0.0 ^b	28.8±1.9 ^b
Untreated	----	4.3±0.1 ^a	-15.0±5.0 ^a	-
MSD (0.05)	----	0.99	19.73	22.52
21DAS				
Botanicals		Ladybird No.	CPD (%)	Reduction (%)
Duduthrin	----	2.0±0.0 ^c	-60.0±0.0 ^c	50.0±0.0 ^a
Nimbecidine	----	3.5±0.1 ^{ab}	-30.0±5.8 ^{ab}	12.5±3.6 ^{bc}
Pyeneem	----	3.0±0.3 ^b	-40.0±8.2 ^b	25.0±5.1 ^b
Pyerin	----	3.0±0.0 ^b	-40.0±0.0 ^b	25.0±0.0 ^b
Untreated	----	4.0±0.0 ^a	-20.0±0.0 ^a	-
MSD (0.05)	----	0.92	18.40	23.00

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

DAS – days after spraying; Pre-spray 1 = spraying at 42 days after planting; Pre-spray 2= Spraying at 70 days after planting; MSD= Minimum significant difference; CPD (%) = change in population density; Reduction (%) = percent reduction in the number ladybird beetles.

Table 5.2 continued....

Botanicals	Without field margin vegetation			
	Second spraying			
	Pre-spray 2	7DAS		
	Ladybird No.	Ladybird No.	CPD (%)	Reduction (%)
Duduthrin	2.8±0.1 ^b	2.0±0.0 ^c	-25.0±8.3 ^b	25.0±4.2 ^a
Nimbecidine	3.5±0.1 ^{ab}	2.8±0.1 ^b	-20.8±7.2 ^{ab}	20.8±3.6 ^{ab}
Pyeneem	3.5±0.1 ^{ab}	2.8±0.1 ^b	-20.8±7.2 ^{ab}	20.8±3.6 ^{ab}
Pyerin	3.0±0.0 ^b	2.0±0.0 ^c	-33.3±0.0 ^b	33.3±0.0 ^a
Untreated	4.0±0.0 ^a	4.0±0.0 ^a	0.0±0.0 ^a	-
MSD (0.05)	0.97	0.61	24.56	24.71
14DAS				
Botanicals		Ladybird No.	CPD (%)	Reduction (%)
Duduthrin	----	2.0±0.0 ^c	-25.0±8.3 ^b	25.0±4.2 ^a
Nimbecidine	----	3.0±0.0 ^b	-12.5±7.2 ^{ab}	12.5±3.6 ^{ab}
Pyeneem	----	3.3±0.1 ^b	-6.3±6.3 ^{ab}	6.3±3.1 ^{ab}
Pyerin	----	3.0±0.0 ^b	0.0±0.0 ^a	0.0±0.0 ^b
Untreated	----	4.0±0.0 ^a	0.0±0.0 ^a	-
MSD (0.05)	----	0.50	22.12	22.12
21DAS				
Botanicals		Ladybird No.	CPD (%)	Reduction (%)
Duduthrin	----	1.5±0.1 ^c	-41.7±16 ^b	43.3±8.4 ^a
Nimbecidine	----	3.5±0.1 ^{ab}	0.0±0.0 ^a	5.0±2.5 ^b
Pyeneem	----	3.5±0.1 ^{ab}	0.0±0.0 ^a	5.0±2.5 ^b
Pyerin	----	3.0±0.0 ^b	0.0±0.0 ^a	5.0±2.5 ^b
Untreated	----	4.3±0.1 ^a	6.3±6.3 ^a	-
MSD (0.05)	----	1.23	36.24	31.73

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

DAS – days after spraying; Pre-spray 1 = spraying at 42 days after planting; Pre-spray 2 = Spraying at 70 days after planting; MSD= Minimum significant difference; CPD (%) = change in population density; Reduction (%) = percent reduction in the number ladybird beetles.

5.4 Discussion

The results from this study have shown that a combination of botanical insecticides and field margin vegetation can significantly enhance the recovery of ladybird beetles compared to synthetic insecticide, Duthrin. The results have further demonstrated that there is a potential for exploiting botanical insecticides and field margin vegetation in conserving key natural enemies such as ladybird beetles. Botanical insecticides have been shown to differ in their mode of action (Stevenson *et al.*, 2017). Natural pyrethrins enter the insect body via ingestion or contact, penetrate the epidermis and are translocated throughout the body in the haemolymph (Fern *et al.*, 2020). Pyrethrins, on the other hand, disrupt the insect's central nervous systems and can also inhibit feeding (Tian *et al.*, 2021). Additionally, neem-based products have been shown to have repellent and antifeedant effects, growth and reproduction inhibition suppressing the insect populations (Cloyd *et al.*, 2009; Dimetry *et al.*, 2010; Isman, 2017). The active components of botanical insecticides are highly labile in ultraviolet light, non-persistent hence the need for integration with other control measures such as biological control (Soares *et al.*, 2019).

In previous studies, botanical insecticides are compatible with other IPM programmes such as entomopathogenic fungi and biological control comprising of predators and parasitoids (Fern *et al.*, 2020; Lavigne *et al.*, 2018). Generalist predators such as aphidophagous coccinellids provide strong suppression of aphids hence the need for their conservation (Scott & Harmon-Threatt, 2021). Planting of field margin vegetation as non-crop plants has 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.

There are successful synergies and compatibilities in integrating botanical insecticides and non-crop habitats for improved insect pest suppression. For instance, Amoabeng *et al.* (2020) demonstrated the potential of exploiting the dual ecosystem services of botanical insecticides and conservation biological control for managing brassicas pests. The study further indicated that integration of non-crop habitat and spraying with either botanical or synthetic insecticide had a strong additive effect against insect pests and their natural enemies. The results from these findings align with what has been found previously that botanical insecticides can be complemented by additional field margin vegetation for reduced disruption of natural enemies (Mkenda *et al.*, 2019).

As a predator of bean aphids, ladybird beetles (Coleoptera: Coccinellidae) can be affected either directly or indirectly by insecticides (Schiess *et al.*, 2017). In a related study, Qin *et al.* (2021) reported decreased feeding activity of ladybird beetles when aphids were treated with azadirachtin. The results indicated that azadirachtin affected the rate at which adult ladybirds preyed on aphids more than larvae, which was attributed to the selectivity of ladybird beetles at different growth stages to the number of chemoreceptors located on the body surface. Fernanda *et al.* (2017) reported high efficacy of azadirachtin against aphids with little effect on egg hatching rate and survival ability of ladybird beetles (*Hippodamia convergens*). Selective insecticides may improve the conservation of natural enemies and therefore, contribute to the success of integrated pest management (IPM) programmes. Results from this study indicated that Nimbecidine had the highest number of ladybird beetles 21 days after the second spraying compared to positive control Duduthrin and the other botanical insecticides (Pyeneem and Pyerin).

The findings of this study have demonstrated that in the presence of field margin vegetation, a higher number of ladybird beetles were recovered compared to the absence of FMV. It was further observed that integration of botanical insecticides and field margin vegetation was more efficient in the recovery of ladybird beetles than the positive control, Duduthrin. Therefore, the integration of field margin vegetation and botanical insecticides can complement IPM in cropping systems that also incorporate flowering plants around field crops to support natural enemies including predators and parasitoids. Adoption of botanical insecticides alongside flowering plant species around crop fields can provide a sustainable and environmentally benign approach to the conservation of ladybird beetles for biological control among the smallholder farmers compared to synthetic insecticides.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General discussion

Synthetic insecticides are known to play a significant role in the management of major insect pests with little regard for beneficial arthropods including predators and parasitoids. Integrated pest management (IPM) programs entail the combination of different control methods to manage insect population and minimise side effects on farmers, beneficial arthropods and the environment (Baker *et al.*, 2020). 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 be conserved with the application of pesticides because of their sensitivity to insecticides as compared to the insect pests (Pezzini & Koch, 2015). Field and cage studies have demonstrated the potential of botanical insecticides and field margin vegetation in reducing bean aphid populations and higher recovery of natural enemies. In addition, in the presence of field margin vegetation, the botanical insecticides produced significantly higher grain yield and above-ground biomass of dolichos that were comparable to the positive control, Duduthrin.

Generally lower aphid abundance, severity and incidence were observed in plots with field margin vegetation. The combination of botanical insecticides and field margins resulted in significantly reduced bean aphid infestation compared to the absence of field margins treated with the insecticides. These results are consistent with Amoabeng *et al.* (2020) who reported high insect pest suppression when botanical insecticides and habitat manipulation were integrated. Non-host plants can, however, reduce insect herbivores' capacity to locate and colonize host plants through chemical and physical interference and this may have also contributed to reducing damage (Mansion-Vaquié *et al.*, 2020). The integration of field margins and synthetic insecticide, Duduthrin (*Lambda-cyhalothrin*), was the most effective treatment for reducing aphid infestation. The compatibility of botanical insecticides with other IPM approaches has also been demonstrated such as with entomopathogenic fungi and biological control comprising of predators and parasitoids (Li *et al.*, 2021). Field margin vegetation has been shown to enhance conservation biological control as the margin plants offer alternative

food resources and this may be a result of the successful synergies and compatibilities of integrating botanical insecticides and non-crop habitats for improved insect pest suppression (Arnold *et al.*, 2021; El-Wakeil, 2014).

The use of insecticides is likely to remain the main component of insect pest suppression in integrated pest management programs; therefore, most studies will be focused on selective insecticides which are compatible with other control measures such as natural pest regulation. Development of new botanical insecticides and selection of the most suitable plant species that support the conservation of natural enemies will to a greater extent boost the food and nutrition security and incomes of the resource-poor smallholder farmers.

6.2 Conclusions

From the results of this study the following conclusions can be made:

- i. Application of commercial botanical insecticides in the presence of field margin vegetation has the potential to reduce bean aphid infestation with negligible effect on natural enemies compared to synthetic insecticide.
- ii. Integration of commercial botanical insecticides and field margin vegetation resulted in higher grain yield and above-ground biomass of dolichos bean.
- iii. Integration of commercial botanical insecticides and field margin vegetation in dolichos bean production had greater efficiency in the recovery of ladybird beetles compared to Duduthrin, a synthetic insecticide.

6.3 Recommendations

- i. It is recommended that smallholder farmers adopt the use of botanical insecticides (Pyeneem & Pyerin) in the presence of field margin vegetation for eco-friendly management of bean aphids and conservation of natural enemies in dolichos bean.
- ii. Further research should be conducted on the viability of integrating botanical insecticides and field margin vegetation for the management of insect pests and improved productivity of legume crops in smallholder agriculture.
- iii. Further studies on a wider range of commercial botanical insecticides and multipurpose flowering plants that can support conservation biological control is recommended.

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APPENDICES

Appendix A: Analysis of variance for the aphid abundance, damage severity and percent incidence on dolichos bean.

Source of variation	df	Aphid abundance	Damage Severity	Incidence (%)
Cropping cycle	1	76.31***	42.64***	4305.62***
Dolichos Growth stage	7	5.09***	4.72***	3203.30***
Cycle× Growth stage	7	0.43***	0.58***	78.92***
Margin Vegetation	1	32.22***	24.57***	15900.15***
Cycle × Margin	1	0.75***	0.28***	288.90***
G. stage × Margin	7	0.29***	0.04	29.35
Insecticide	4	22.48***	14.51***	5319.62***
Cycle × Insecticide	4	1.54***	1.26***	83.26***
G. Stage × Insecticide	28	0.67***	0.58***	204.74***
Margin × Insecticide	4	0.25***	0.09**	12.16
G. stage× Margin × Insecticide	28	0.02	0.02	24.28*
Cycle × G. stage× Margin × Insecticide	67	0.06***	0.05***	16.62
Replication	3	0.00	0.07	31.97
Error	477	0.02	0.02	15.18
CV (%)		5.79	7.41	7.51
R²		0.97	0.94	0.91

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

Appendix B: Combined mean comparison for aphid abundance, damage severity and percent incidence on dolichos bean as influenced by selected botanical insecticides and field margin vegetation over two cropping cycles (May-December 2019 and March-November 2020).

Cropping cycle	FMV	Treatment	Aphid abundance	Damage Severity	Incidence (%)
2019	absent	Untreated	2.675 c	2.453 c	64.688 ab
		Nimbecidine	2.044 ef	2.003 efg	53.594 de
		Pyeneem	1.866 fgh	1.816 gh	51.563 e
		Pyerin	1.963 fg	1.931 fg	51.875 e
		Duduthrin	1.803 ghi	1.834 gh	46.250 fg
	present	Untreated	2.178 de	2.041 def	54.844 de
		Nimbecidine	1.709 hij	1.691 hi	45.469 g
		Pyeneem	1.553 jk	1.491 j	43.438 g
		Pyerin	1.597 ijk	1.581 ij	43.125 g
		Duduthrin	1.413 k	1.488 j	37.969 h
2020	absent	Untreated	2.856 bc	2.556 bc	57.500 cd
		Nimbecidine	3.006 b	2.663 b	60.625 bc
		Pyeneem	2.381 d	2.131 de	52.344 e
		Pyerin	2.319 d	2.156 de	50.469 ef
		Duduthrin	2.291 d	2.156 de	50.469 ef
	present	Untreated	2.341 d	2.203 d	52.031 e
		Nimbecidine	2.347 d	2.131 de	52.031 e
		Pyeneem	2.284 d	2.172 de	50.781 e
		Pyerin	3.494 a	3.153 a	65.938 a
		Duduthrin	2.388 d	2.169 de	52.500 e

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

Appendix C: Analysis of variance for the abundance of key natural enemy species found on dolichos bean.

Source of variation	df	Parasitic wasps	Tachinid flies	Ladybird beetles
Cycle	1	140.53***	143.26***	6.72***
Dolichos growth stage	3	3.00***	6.20***	3.51***
Cycle × G. stage	3	2.45***	2.74***	0.49**
Margin vegetation	1	17.25***	22.65***	1.05***
Cycle × Margin	1	5.53***	8.46***	1.66***
G. stage × Margin	3	0.41**	0.95***	0.08
Trapping area	1	86.50***	47.52***	3.30***
Cycle × T. area	1	63.81***	62.50***	6.52***
Insecticide	4	3.15***	4.24***	2.16***
Margin × Insecticide	4	0.05	0.08	0.07
Cycle × Insecticide	4	0.09	0.21	0.21*
G. stage × Insecticide	12	0.40***	0.42***	0.24**
G. stage × T. area × Insecticide	19	0.31***	0.22*	0.08
G. stage × Margin × Insecticide	12	0.11	0.03	0.04
Replication	3	0.00	0.05	0.04
Error	567	58.83	68.92	52.82
CV		15.07	15.86	18.95
R²		0.85	0.83	0.46

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

Appendix D: Combined mean comparison for the abundance of parasitic wasps, tachinid flies and ladybird beetles as affected by selected botanical insecticides and field margin vegetation over two cropping cycles (May-December 2019 and March-November 2020).

Cropping cycle	FMV	Treatment	Parasitic wasps	Tachinid flies	Ladybird beetles
2019	FMV absent	Untreated	2.406 fg	2.625 hi	2.375 bc
		Nimbecidine	1.813 fg	1.781 i	1.844 cdef
		Pyeneem	1.531 g	2.219 hi	1.969 bcde
		Pyerin	1.500 g	1.719 i	1.625 defg
		Duduthrin	1.313 g	1.188 i	1.156 g
	FMV present	Untreated	3.094 ef	3.500 gh	3.656 a
		Nimbecidine	2.594 fg	2.500 hi	2.250 bcd
		Pyeneem	2.063 fg	2.406 hi	2.531 b
		Pyerin	1.906 fg	2.219 hi	2.437 bc
		Duduthrin	1.375 g	1.750 i	1.563 efg
2020	FMV absent	Untreated	6.719 bc	6.969 cd	2.000 bcde
		Nimbecidine	5.562 cd	5.687 de	1.500 efg
		Pyeneem	5.406 cd	5.187 ef	1.625 defg
		Pyerin	5.062 d	5.188 ef	1.719 defg
		Duduthrin	4.500 de	4.156 fg	1.156 g
	FMV present	Untreated	9.719 a	10.625 a	1.875 cdef
		Nimbecidine	7.500 b	8.500 b	1.469 efg
		Pyeneem	7.687 b	7.687 bc	1.250 fg
		Pyerin	7.156 b	8.094 bc	1.125 g
		Duduthrin	6.719 bc	6.875 cd	1.125 g

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

Appendix E: Analysis of variance for growth and yield components in dolichos bean.

Source of variation	df	PH (m)	UDPP	DPP	Seeds/pod	100SW (g)	GY (tons/ha)	Biomass (tons/ha)
Cycle	1	0.57***	2142.45***	348.61***	0.30**	0.00	5.77***	7.64***
Insecticide	4	0.43***	469.35***	524.30***	0.16**	24.70***	5.12***	26.85***
Cycle*Insecticide	4	0.14***	57.16***	21.73***	0.03	11.75***	0.34***	5.54***
Margin	1	1.68***	2668.05***	588.61***	0.82***	115.72***	3.48***	130.89***
Cycle*Margin	1	0.16**	696.20***	4.51*	0.03	17.37***	0.00	0.04
Margin*Insecticide	4	0.01	17.58***	26.30***	0.05	1.53*	0.15***	1.24***
Cycle*Margin*Insecticide	4	0.07**	34.91***	2.76*	0.05	5.03***	0.05***	0.05
Replication	3	0.02	0.63	0.64	0.06	0.63	0.00	0.04
Error	57	1.27	1.70	0.82	0.03	0.63	0.00	0.05
CV (%)		15.01	4.27	4.49	5.28	3.11	2.55	3.31
R²		0.80	0.98	0.90	0.56	0.89	0.99	0.98

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

UDPP= Undamaged pods per plant, DPP = Damaged pods per plant, 100SW=100 seed weight (g), GY = Grain yield (tons/ha), PH = Plant height (m).

Appendix F: Combined mean comparison for undamaged pods/plant, damaged pod/plant, seeds/pod, 100seedweight, grain yield, biomass and plant height as influenced by selected botanical insecticides and field margin vegetation over two cropping cycles (May-December 2019 and March-November 2020).

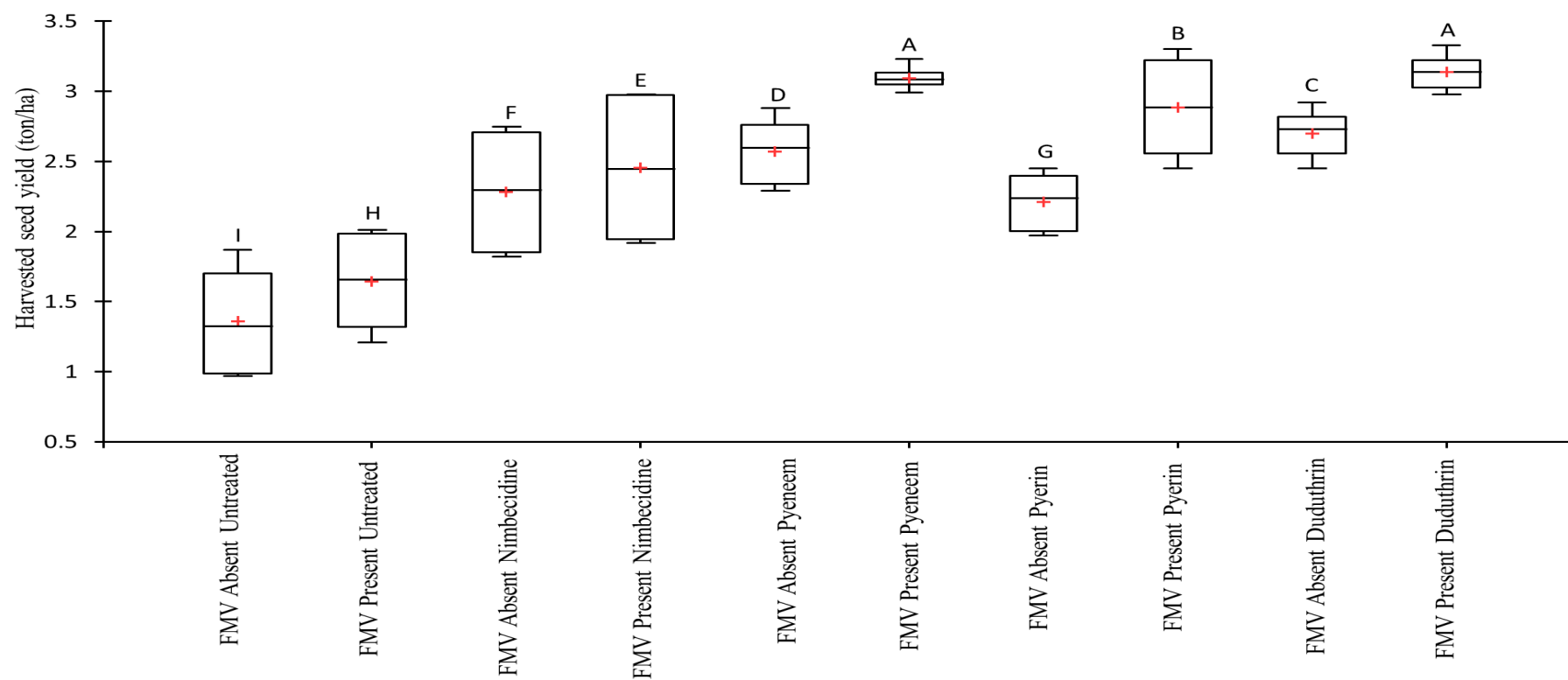
Cropping cycle	FMV	Treatment	PH (m)	UDPP	DPP	Seeds/pod	100SW (g)	GY (tons/ha)	Biomass (tons/ha)
2019	absent	Untreated	0.668 fg	15.750 m	33.500 b	3.375 def	20.200 g	0.990 m	5.452 k
		Nimbecidine	0.983 bcde	19.500 l	21.750 de	3.550 bcd	24.963 cd	1.855 j	6.468 hi
		Pyeneem	1.015 bcde	25.500 ij	16.000 h	3.650 abc	25.503 bcd	2.353 h	6.450 hi
		Pyerin	0.815 def	26.000 ij	16.000 h	3.700 abc	22.538 f	2.014 i	5.095 l
		Duduthrin	0.967 bcde	26.000 ij	15.500 h	3.450 cde	26.513 b	2.553 g	8.243 e
	present	Untreated	0.805 ef	23.000 k	25.000 c	3.575 bcd	24.918 d	1.293 l	7.238 g
		Nimbecidine	1.558 a	26.500 hi	18.500 f	3.675 abc	26.600 b	1.954 i	8.835 d
		Pyeneem	1.538 a	28.750 fg	11.250 ij	3.850 a	27.918 a	3.045 c	9.843 b
		Pyerin	1.005 bcde	28.250 gh	12.500 i	3.675 abc	27.980 a	2.550 g	7.763 f
		Duduthrin	1.443 a	34.500 d	10.750 j	3.750 ab	28.988 a	3.040 c	11.045 a

Means in a column followed by the same letters are not significantly different at $P<0.05$ using Tukey's HSD test; UDPP= Undamaged pods per plant, DPP = Damaged pods per plant, 100SW=100 seed weight (g), GY = Grain yield (tons/ha), PH = Plant height (m)

Appendix F continued...

Cropping cycle	FMV	Treatment	PH (m)	UDPP	DPP	Seeds/pod	100SW (g)	GY (tons/ha)	Biomass (tons/ha)
2020	absent	Untreated	0.550 g	19.000 l	35.750 a	3.175 f	25.095 cd	1.728 k	3.467 m
		Nimbecidine	0.828 cdef	29.750 fg	25.250 c	3.650 abc	23.763 e	2.711 f	6.071 j
		Pyeneem	0.943 bcde	29.750 fg	21.500 de	3.475 cde	24.768 de	2.790 ef	6.405 hij
		Pyerin	0.871 cdef	24.250 jk	22.750 d	3.250 ef	25.120 cd	2.410 h	6.240 ij
		Duduthrin	0.854 cdef	32.250 e	20.750 e	3.350 def	25.613 bcd	2.845 e	6.658 h
	present	Untreated	0.881 cde	30.500 ef	24.250 c	3.450 cde	25.730 bcd	1.990 i	5.492 k
		Nimbecidine	0.998 bcde	44.000 c	20.500 e	3.550 bcd	25.215 cd	2.952 d	8.073 ef
		Pyeneem	1.040 bc	49.500 b	17.750 fg	3.700 abc	26.530 b	3.141 b	9.710 bc
		Pyerin	1.103 b	44.500 c	17.500 fg	3.675 abc	26.050 bc	3.221 ab	8.698 d
		Duduthrin	1.026 bcd	53.750 a	16.500 gh	3.750 ab	28.200 a	3.235 a	9.435 c

Means in a column followed by the same letters are not significantly different at $P < 0.05$ using Tukey's HSD test; UDPP= Undamaged pods per plant, DPP = Damaged pods per plant, 100SW=100 seed weight (g), GY = Grain yield (tons/ha), PH = Plant height (m).



Appendix G: Dolichos bean yield from botanical insecticides, Duduthrin and untreated when cropped with a field margin vegetation present or absent.

Box plots are at 95% confidence intervals where + is the mean, the central black line the median, upper 1st and lower 3rd quartiles and whiskers for max/min values. Letters above each box plot are from a post-hoc Least Significant Difference test showing differences in mean values at the 95% confidence interval.

Appendix H: Analysis of variance for population densities of ladybird beetles on dolichos bean as influenced by botanical insecticides and field margin vegetation.

Source of variation	df	Ladybird No.	Ladybird CPD (%)	Ladybird Reduction (%)
Treatment	4	52.77***	6747.77***	6945.63***
Field margin vegetation	1	91.37***	22.57	1302.49***
Dolichos growth stage	7	14.43***	7220.32***	4878.22***
Replication	3	0.61***	657.05***	254.19***
Treatment × FMV	4	5.30***	651.80***	699.8***
Treatment × G. stage	28	2.04***	550.23***	588.39***
FMV × G.stage	7	7.52***	800.81***	559.87***
Treatment × FMV × G. stage	28	0.95***	129.84	117.79
Error	237	0.19	85.12	82.29
CV (%)		10.98	-55.28	20.13
R²		0.93	0.84	0.82

***, significant at ($P \leq 0.001$); CV=Coefficient of variation; d.f= degree of freedom

CPD (%) = change in population density of ladybird beetles.

Appendix I: Combined mean comparison for population densities of ladybird beetles on dolichos bean as influenced by botanical insecticides in the presence of field margin vegetation.

Field margin vegetation	Treatment	Ladybird No.	Ladybird CPD (%)	Ladybird Reduction (%)
FMV present	Duduthrin	3.344 ef	-25.000 f	26.401 b
	Pyeneem	4.438 b	-20.737 def	21.741 c
	Pyerin	4.281 b	-17.917 de	19.786 cd
	Nimbecidine	3.906 c	-21.302 ef	22.958 bc
	Untreated	6.406 a	2.232 a	-
FMV absent	Duduthrin	2.438 g	-33.333 g	31.354 a
	Pyeneem	3.656 d	-12.917 c	9.635 f
	Pyerin	3.469 de	-16.510 cd	13.854 ef
	Nimbecidine	3.156 f	-18.542 de	15.885 de
	Untreated	4.313 b	-3.594 b	-

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

Appendix J: Author's publications

i. Publications in Refereed Scientific Journals

Ochieng, L. O., Ogendo, J. O., Bett, P. K., Nyaanga, J. G., Cheruiyot, E. K., Mulwa, R. M., Arnold, S. E., Belmain, S. R., Stevenson, P. C. (2022). Field margins and botanical insecticides enhance Lablab purpureus yield by reducing aphid pests and supporting natural enemies. *Journal of Applied Entomology*, 00, 1–12. <https://doi.org/10.1111/jen.13023>

Received: 19 December 2021 | Revised: 21 April 2022 | Accepted: 28 April 2022
DOI: 10.1111/jen.13023

ORIGINAL CONTRIBUTION

JOURNAL OF APPLIED ENTOMOLOGY WILEY

Field margins and botanical insecticides enhance *Lablab purpureus* yield by reducing aphid pests and supporting natural enemies

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Funding Information

Grand Challenges Research Fund (GBRC), Grant/Award Number: GB/R020561/1

Abstract

Botanical insecticides offer an environmentally benign insect pest management option for field crops with reduced impacts on natural enemies of pests and pollinators while botanically rich field margins can augment their abundance. Here, we evaluated the non-target effects on natural enemies and pest control efficacy on bean aphids in Lablab of three neem- and pyrethrum-based botanical insecticides (Pyrin75EC®, Nimbecidine® and Pyeneem 20EC®) and determine the influence of florally rich field margin vegetation on the recovery of beneficial insects after treatment. The botanical insecticides were applied at the early and late vegetative growth stages. Data were collected on aphids (abundance, damage severity and percent incidence) and natural enemy (abundance) both at pre-spraying and post-spraying alongside Lablab bean yield. The efficacy of botanical insecticides was similar to a synthetic pesticide control and reduced aphid abundance by 88% compared with the untreated control. However, the number of natural enemies was 34% higher in botanical insecticide-treated plots than in plots treated with synthetic insecticide indicating that plant-based treatments were less harmful to beneficial insects. The presence of field margin vegetation increased further the number of parasitic wasps and tachinid flies by 16% and 20%, respectively. This indicated that non-crop habitats can enhance recovery in beneficial insect populations and that botanical insecticides integrate effectively with conservation biological control strategies. Higher grain yields of 2.55–3.04 and 2.95–3.23 t/ha were recorded for both botanical insecticide and synthetic insecticide in the presence of florally enhanced field margins in consecutive cropping seasons. Overall, these data demonstrated that commercial botanical insecticides together with florally rich field margins offer an integrated, environmentally benign and sustainable alternative to synthetic insecticides for insect pest management and increased productivity of the orphan crop legume, Lablab.

ii Publications in Refereed Conference Proceedings

Ochieng, O. L., Ogendo, O. J., Bett, K. P., J. Nyaanga G.J., Cheruiyot E. & Mulwa, S. R (2020). *Influence of botanical insecticides and field margin vegetation on aphids and their natural enemy abundance in Dolichos bean (Loblob purpureus L.)*. Book of abstracts, Proc. 13th Biennial Egerton University International Virtual Conference: Innovation, Research and Transformation for Sustainable Development, 24th – 26th November 2020. pp.67

Abstract

Influence of Botanical Insecticides and Field Margin Vegetation on Aphids and their Natural Enemy Abundance in Dolichos Bean (*Loblob purpureus* L.)

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and R. M.S. Mulwa¹**

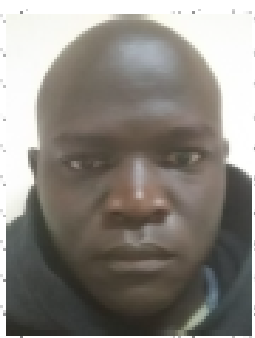
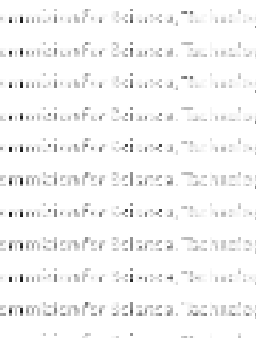
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Insecticides of plant origin have been reported to be less harmful to the environment and beneficial insects. However, the effect of botanical insecticides on insect pests and their natural enemies in *Dolichos* bean (*Loblob purpureus* L.) under diverse environments is not well understood. A study was conducted to determine the effect of botanical insecticides and field margins on aphids and their natural enemies' abundance in *Dolichos* bean. Five treatments comprising three commercial botanical insecticides (Pyerin75EC[®], Nimbecidine[®] and Pyeneem 20EC[®]), a synthetic insecticide (Duduthrin 1.75EC[®]) and an untreated control were laid out in a randomized complete block design (RCBD) with four replicates either in the presence or absence of field margin plant species. Aphids' abundance, incidence and severity were influenced by the crop growth stage, treatment applied and presence or absence of field margins. Highest aphid abundance (2.1), severity (2.68) and percent incidence (52.92%) were recorded on untreated plots. The synthetic insecticide, Duduthrin, showed reduced levels of aphid infestation which was not significantly different from Pyeneem ($P < 0.05$). However, Duduthrin had a significant reduction in natural enemy populations which are natural biological control agents for bean aphids. Results showed that field margin plant species attracted parasitic wasps (56%), tachinid flies (56%) and Coccinellid beetles (58%). Of the 684 Coccinellid beetles that were collected, 397 were from plots with field margin species, showing that they had the greatest abundance. These findings have demonstrated that conservation of field margin plant species and use of botanical insecticides hold good promise as ecologically benign and viable alternatives to synthetic insecticides in the management of aphids in *Dolichos* beans.

Keywords: Aphids, field margins, *Loblob purpureus*, natural enemies,

Appendix K: Research permit

			
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