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Enhancement of plant extracts use for pest control and growth promotion of common bean (Phaseolus vulgaris)

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ENHANCEMENT OF PLANT EXTRACTS USE FOR PEST CONTROL AND GROWTH PROMOTION OF COMMON BEAN (*Phaseolus vulgaris*)

Angela Gerald Mkindi

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

For smallholder farmers, suitable plants for pest management and as foliar feed are obtained with ease, and when successfully exploited, could contribute to local income generation through commercialization. However, with extensive research on their efficacy, toxicity and availability, the use of plant extracts is not widely adopted especially for smallholder farmers in rural settings. This study focused on evaluating factors that can foster extensive use of plant extracts among smallholder farmers. Questionnaires and focus group discussion were used to assess the perception of farmers towards using pesticidal plants, highlighting possible challenges, benefits and future enabling aspects for sustainable bean crop production. Plots of $5m^2$ were established by farmers where an evaluation of the efficacy of *Tephrosia vogelii, Tithonia diversifolia* and *Lantana camara* was done to ascertain their potential for common bean insect pest management and impacts on beneficial arthropods. Additionally, the study evaluated spatio-temporal variability in bioactive phytochemicals of the most effective plant (*T. vogelii*), as well as the contribution of *T. vogelii* and *T. diversifolia* towards growth promotion and yield of common beans. Results showed that high per cent (99%, n=67) of smallholder farmers had pest challenge and that only (39.7%, n= 27) reported using plant extracts. Likewise, farmers reported a lack of working tools and motivation from researchers and extension officers as a challenge hindering the use of plant extracts. Plant extracts showed efficacy in pest management compared with untreated control whereby *T. vogelii* significantly reduced abundance of aphids (0.06 ± 0.02) and foliage beetles (0.17 ± 0.03 compared with untreated (0.4 ± 0.05 and 0.5 ± 0.04 respectively). Again, the increased grain yield was recorded on plots treated with *T. vogelii* (3.8 ± 0.23) and *T. diversifolia* (3.3 ± 0.23) compared with untreated beans (1.5 ± 0.16), when applied as a foliar spray (2.7 ± 0.20) compared with soil drench (2.1 ± 0.16). Phytochemical variation was noted in *T. vogelii* where an additional chemotype 3 was first recorded. Hence, under smallholder farming conditions, plant extracts can contribute to sustainable bean crop production if practical implementation that involves smallholder farmers is a priority.

DECLARATION

I, **ANGELA GERALD MKINDI** do hereby declare to the Senate of Nelson Mandela African Institute of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

Angela Gerald Mkindi 28/04/2016
Name and signature of candidate **Date**

The above declaration is confirmed

Prof. Patrick A Ndakidemi 2nd MAY 2016
Name and signature of supervisor **Date**

Prof. Karoli N Njau 9 May 2016
Name and signature of supervisor **Date**

Dr. Kelvin M Mtei April 29th 2016
Name and signature of supervisor **Date**

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CERTIFIATION

This is to certify that the accompanying dissertation by ANGELA GERALD MKINDI has been accepted in partial fulfilment of the requirements for the Degree of Environmental science and Engineering of the Nelson Mandela African Institution of Science and Technology Arusha, Tanzania.

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DEDICATION

This dissertation is dedicated to my children, Ivan and Joan. I love you, and I pray that God bless each of you and that this work inspires you positively, to work hard, be honest, to have positive attitudes towards life and all it brings, see opportunities and work towards them, be dedicated and achieve much more than this.

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LIST OF ABBREVIATIONS AND SYMBOLS

AN	Anthocyanins
ANOVA	Analysis of Variance
BC	Before Christ
CC	Chlorophyll Content
CREATES	Centre for Research, Agricultural advancement, Teaching Excellence and Sustainability
DDT	Dichlorodiphenyltrichloroethane
DMSO	Dimethylsulfoxide
ESIMS	Electrospray Ionization Mass Spectroscopy
FAO	Food and Agriculture Organization of the United Nations
FFS	Farmer Field Schools
FGD	Focus Group Discussion
FL	Flavonoids
FRN	Farmer Research Network
FS	Folia spray
GIS	Geographic Information System
HPLC	High-Performance Liquid Chromatography
LA	Leaf Area
LC-MS	Liquid chromatography-mass spectrometry
LG	Leaf Greenness
LSD	Least Significant Difference
MS	Molecular Mass
NB	Number of Branches
NL	Number of Leaves
NM-AIST	The Nelson Mandela African Institution of Science and Technology
NPP	Number of Pods per plant
PCA	Principal Component Analysis
PDA	Photodiode Array
PH	Plant Height
Phen	Phenylalanine
Ru	Rutin
SD	Soil drench
SE	Standard Error
SPSS	Statistical Package for Social Sciences
SW	Stem Width
SY	Seed yield
TMA	Tanzania Meteorological Agency
Trypt	Tryptophan
UV	Ultra Violet
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the Problem

Smallholder common bean (*Phaseolus vulgaris*) farming communities face a prominent challenge of insect pests that leads to reduced production and poor quality crops (Hillocks *et al.,* 2006). Using synthetic insecticide formulations is a pest management approach which poses problems because they are not always affordable by local farmers in addition to having health and environmental impacts (Isman & Grieneisen, 2014). Harmfulness of synthetic chemicals can be explained better by the restriction of DDT in the 1970s because of identified persistence of residues in the environment and human bodies (Beard, 2006). As a result, literature reports banning of DDT in Europe along with other synthetic chemicals through the pesticide regulation (EC) No. 1107/2009 (Villaverde *et al.,* 2014). In addition to banning synthetic chemicals, such agencies encourage biopesticides as alternative options (Villaverde *et al.,* 2014). Africa, on the other hand, appears to be as an appropriate place for emphasizing the use of plant extracts as cheap and alternative to synthetic chemicals (Isman, 2006; 2020) because of the existing diversity of plant species, many of which bearing pesticidal properties. However, the adoption of plant extract use in developing countries is low, despite the presence of high diversity of pesticidal plants species in local farming areas (Williams *et al.*, 2000). While plant extracts are underutilized, pest outbreaks, poor soils and poor bean growth conditions force farmers to use fertilizers and synthetic pesticides in higher quantities, causing adverse impacts. Hence, a safe, affordable and available alternative such as using plant extracts is highly required.

Research outputs since 1980s have shown potential efficacy of plant products for field and storage pests control, thus still motivating its use as a safer option compared with synthetic pesticides (Isman & Grieneisen, 2014). In addition to efficacy studies (Isman, 2006), investigations on potential risks show fewer hazards to environment and humans (Isman, 1997; Mkenda *et al.,* 2015; Mkindi *et al.*, 2017; Tembo *et al.*, 2018). Based on such benefits, some plant extracts such as neem with its azadirachtin active compound are popular because of the extensive research on the efficacy, that facilitates massive growth, harvesting and processing (Koul *et al.,* 2000). Other plant extracts may not be as popular and commercial as neem, partly because of the pressure from using synthetic pesticides formulation, which suppresses awareness, and motivation towards using them.

Extracts from a plant such as neem are known to provide for foliar plants nutrition and systemic resistance and hence improve crop plant growth (Pretali *et al.,* 2016). Other plant species useful for pest management such as *Tephrosia vogelii* and *Tithonia diversifolia* are known to increase soil nutrients (Jama *et al.*, 2000; Mafongoya *et al.*, 2003; Munthali *et al.*, 2014). Research works providing scientific evidence for the use of such plants as foliar fertilizer are essential to make plants known for multiple functions, including pest management as well as foliar nutrients supply. As a result, understanding the different functions of plant extracts in crop production would enhance commercialization and broaden the uses by farmers as envisaged by Mkindi *et al.* (2017).

1.2 Statement of the Problem

The use of pesticidal plants is a good alternative in crop production, particularly beans (Mkenda *et al.*, 2015; Mkindi *et al.*, 2017; Tembo *et al.*, 2018). Some terrestrial plants produce chemicals that are as effective as broad-spectrum pesticides against field insect pests as well as promoting the growth of food crops (Jama *et al.*, 2000; Mafongoya *et al.*, 2003; Munthali *et al.*, 2014; Pretali *et al.,* 2016). Studies from Africa show the vast diversity of pesticidal plant species suitable for pest management (Isman, 2020). However, most findings are under laboratory and controlled experimental conditions which have less reflection to field situations from where agronomic challenges originate. Some of the challenges include; unreliable raw materials supply and traditional variable methods of preparation that lead to inconsistent efficacy and existence of inherent differences in plant chemistries. The effectiveness of home-prepared concoctions based on plants is variable, as there are unavoidable variations in the raw material. Hence this study attempts to establish a practical strategy towards plant extracts use by investigating factors affecting adoption, analysis of the the spatio-temporal variation of active compounds as well as a study of the multiple functions of plant extracts including pest management, impacts to beneficial arthropods and growth promotion.

1.3 Rationale of the Study

Generally, the importance of common beans in nutrition and economy of smallholder farmers is crucial (Hillocks *et al.,* 2006). Being produced primarily by resource-poor communities, common bean production requires considerations in using affordable but safe and effective approaches that ensure cost-effective production (Mkindi *et al.*, 2017). Investigations of several plant species in sub-Saharan Africa have shown their usefulness by availing pest management potentials and yield increase. With evidence of efficacy under local natural condition (Sola *et al.*, 2014; Mkenda *et al.*, 2015; Mkindi *et al.*, 2017) less has been done to catalyze their extensive use by smallholder farming communities from where bean production challenge is significant. Apart from research on pesticidal plants extracts, the different ways by which plant extracts can be used are of paramount importance because farmers know the plants for their pest control and growth promotion qualities (Dougoud *et al.,* 2019). Importantly pesticidal plants are readily available and affordable to low-income farmers for practical pest management (Mkenda *et al.*, 2015; Mkindi *et al.*, 2017; Tembo *et al.*, 2018). Also, elderly farmers have used such technology for generations, making it more acceptable and trusted. Farmers involvement in research and evaluation of the efficacy of plant extracts are required to enhance maximum participation and collaborative innovation. Hence, this study evaluated the feasibility of using pesticidal plants by smallholder farmers in four main aspects; (a) examining the possible challenges and benefits associated with the use of plant extracts locally as well as future plans towards extensive use of the technology (b) analyzing the spatial-temporal chemical variation of the most used plant species, *T. vogelii* (c) establishing collaborative trials to evaluate the efficacy of extracts on the field, led by smallholder farmers and (d) to determine the use of plant extracts for growth promotion. Fulfilment of this work motivates the use of plant extracts by farmers through providing practical and influential information for farmers that add value to bean production systems.

1.4 Objectives

1.4.1 Main Objective

To enhance plants extracts use for pest control and growth promotion in common beans (*Phaseolus vulgaris*).

1.4.2 Specific Objectives

(i) To assess the social implications “challenges, benefits and ways forward” of the current adoption of pesticidal plant formulations by smallholder farmers.

(ii) To evaluate pesticidal plants efficacy on common bean insect pest control by using a Farmer Research Network design.

(iii) To assess the spatio-temporal variation of phytochemicals in *T. vogelii*.

(iv) To evaluate the contribution of pesticidal plant extracts on bean plant metabolites and grain yield.

1.5 Research Hypothesis

(i) Farmers' understand drivers for plants extracts use for sustainable bean crop production.

(ii) Pesticidal plants extracts applied under natural field conditions are adequate for insect pest control and favour the presence of beneficial arthropods.

(iii) Bioactive chemicals in pesticidal plants vary across space and time and may influence farmers' use.

(iv) There is a significant contribution from sprayed extracts of *T. diversifolia* and *T. vogelii* to the growth and grain yield of common beans.

1.6 Significance of the Study

The study will increase an understanding of factors that influence the use of plant extracts among smallholder farmers. It will also facilitate collaboration between researchers and farmers in evaluating plant extracts, hence motivating joint innovation in natural products used for crop production. Through this study, bean producers will benefit from a clearer understanding of challenges, benefits and ways forward towards using plant extracts, which would inform policymakers on sustainable pest management options. Of greater importance, enhanced awareness of plant extracts and their benefits would contribute to local commercialization, thereby contributing to local farmers' economic wellbeing.

1.7 Delineation of the Study

This study was conducted to enhance the use of plant extracts among smallholder farmers involving main aspects as follows:

(i) The collaborative assessment of factors that can influence the uptake of plant extracts uses among 81 smallholder farmers.

(ii) Joint experimentation of efficacy of plant extracts on common bean pests, effects on beneficial arthropods, damage and yield of common beans among 100 farmers.

(iii) Evaluation spatio-temporal variation of chemotypes in *T. vogelii* that was conducted in Kenya, Tanzania and Malawi.

(iv) Evaluation of the contribution of plant extracts on bean growth, metabolites production and grain yield.

(v) Analyses of samples for metabolites in common beans and phytochemicals in *T. vogelii* were carried out in two laboratories namely the Nelson Mandela African Institution of Science and Technology (NM-AIST) and Jodrell Laboratory in KEW United Kingdom.

CHAPTER TWO

LITERATURE REVIEW

2.1 Background of Local Plant Extracts Use

Use of plant extracts for pest control dates back to the second millennium BC, where the use of poisonous plants for pest control existed in a prominent Indian book known as *Rig Veda* (Biswas, 2009). Aromatic plants such as rosemary were used as fumigants, hung near granaries to repel insect pests (Pavela, 2016). Nicotine from tobacco plant was the first insecticide in the 17th century used to control plum beetles (Pavela, 2007). However, nicotine is currently discouraged because of adverse health impacts (Price & Martinez, 2019). Additionally, the first use of pyrethrins as insecticides was since 400BC (Ensley, 2018). Rotenoids use dates back to the 1850s known as a fish poison, collected from the plant known as Timbo (Pavela, 2007). This brief history signifies the importance of plants to humankind and their impacts on pest management. In the 19th century, with the advancement in plants extracts knowledge, using pesticidal plants entered in a phase of research where scientific validation of activity, documenting potential benefits to the ecosystem and human beings (Tiilikkala *et al.,* 2011) was undertaken. Knowledge about using plant extracts in ancient time is crucial because it sets the basis for current efforts towards facilitating the more extensive use of plant extracts in the world. However, with such history, the use of plant extracts is still inadequately explored among farming communities despite massive research that is conducted. It is therefore important to explore factors that influence adoption, acceptance and use of such plants especially by smallholder farmers.

2.2 Benefits of Using Plant Extracts

2.2.1 Availability of Plants Species

Use of plant extracts is majorly practised by subsistence to transitional farmers (Dougoud *et al.,* 2019). The main motive behind using plant extracts is their availability where plant materials can be harvested easily, whenever labour is enough (Amoabeng *et al.,* 2014; Cosmas *et al.,* 2012). Plants extracts can be locally processed and applied, demanding less sophisticated machinery and also using indigenous knowledge that exists among smallholder farmers meanwhile requiring less precision (Isman, 2008; Mihale *et al.,* 2009).

Plant species such as *T. diversifolia*, *L. camara* and *T. vogelii* are among good examples of readily available pesticidal plant species found in farmers' vicinity. These species have been used for evaluating efficacy against insect pests in maize/common beans systems under local conditions and have shown promising pest control and grain yield increase results (Mkindi *et al.,* 2017; Tembo *et al.,* 2018). Pesticidal plants materials such as *T. diversifolia* and *L. camara* thrive on roadsides, abandoned fields and in field margins making their availability sure in the majority of smallholder farming community's neighbourhoods. Use of such plants in crop production would slow their spread speed, hence serving as a beneficial suppression strategy (Kannan *et al.,* 2016). On the other hand, *T. vogelii*, is a native of tropical Africa, non-invasive, drought-tolerant, and so can be propagated easily by smallholder farmers.

2.2.2 Multiple Uses of Pesticidal Plant Species

A pesticidal plant such as *T. diversifolia* is known for various applications including pest control, human disease cure and soil enrichment (Mkenda *et al.,* 2015: Olowokere, & Odulate, 2019). In Africa, predictions of the spread of *T. diversifolia* show that it has spread in the East, Central and West Africa, some parts of South Africa and Madagascar (Obiakara & Fourcade, 2018). Apart from its invasive nature, local use of the plant spans from the treatment of ailments (malaria, diabetes and snake bites) and to agricultural benefits (soil enrichment and pest control) (Ajao & Moteetee, 2017). In soil enrichments, *T. diversifolia* can enhance symbiosis with native mycorrhiza for phosphorus absorption (Scrase *et al.,* 2019). Studies have shown that negative impacts of *T. diversifolia* on invasiveness are not established; hence the beneficial contributions are more than the destructive invasive shortcomings (Witt *et al.,* 2019).

Another widespread pesticidal plant which contains invasive traits by dominating the understory and reducing nutrients for other plants, as well as assemblages for other organisms, is *L. camara* (Gooden *et al.,* 2009; Osunkoya & Perrett, 2011; Jambhekar & Isvaran, 2016). In Eastern Africa, *L. camara* is a known threat, encroaching crops and pasture lands (Shackleton *et al.,* 2017). On the other hand, *L. camara* is also a known biofumigant having a neurotoxic mode of action used in postharvest pest control (Zandi-Sohani & Khuzestan, 2012; Rajashekar *et al.,* 2014).

Field trials have shown the contribution of locally processed *L. camara* for pest control in common beans and for their fewer effects on beneficial insects (Mkindi *et al.*, 2017). Ecologically, *L. camara* is useful in soil fertility improvement through the biomass, soil and water retention, and harbouring beneficial insects (Negi *et al.*, 2019).

Additionally, *T. vogelii* is a multipurpose plant used for pest control and soil enrichment (Paramu *et al.*, 2005). It is a native of Africa and can grow from seeds (Dzenda *et al.*, 2008). This shrub can be intercropped with crop plants such as maize (Gachene & Wortmann, 2006; Gilbert, 2006), used for improved fallows (Mafongoya *et al.*, 2003) and it is an insecticide (Belmain *et al.*, 2012; Stevenson *et al.*, 2012; Mkindi *et al.*, 2017; Stevenson *et al.*, 2017; Kayange *et al.*, 2019). Being non-invasive and indigenous, *T. vogelii* could fit as a nonfood crop to farmers who would opt to propagate and use in crop production due to its multiple uses. A recent study by Mkindi *et al.* (2020) showed the contribution of *T. diversifolia* and *T. vogelii* into grain yield increase and metabolites production through foliar application of extract, signifying an added advantage of plant extracts for growth promotion.

2.2.3 Safety of Plant Extracts

Plant extracts are known to have fewer health risks compared with synthetic formulations and are compatible with ecological functioning (Sola *et al.*, 2014). Their use has motivated local as well as international agencies having concern on agricultural sustainability (Isman, 2015). Tembo *et al.* (2018) demonstrated fewer risks to humans and the environment when using locally processed plant extracts signifying that crude extracts contain minimal concentrations of compounds ensuring minimum health and environmental risks (Isman, 2008; Khater, 2012). Pesticidal plant species are known to have varied modes of actions, based on their phytochemical composition which could delay resistance building in target insect pests (Derbalah *et al.*, 2012) hence leading to efficient pest management. Likewise, compounds of natural plants origin are thermo- or UV-labile, therefore existing for a short period in the crop and the environment hence ensuring that foods are free from residues (Pavela & Benelli, 2016). Plants including pesticidal plants harbour other insect species such as natural enemies and pollinators by providing forage and breeding sites (Tembo *et al.*, 2018); as a result, increasing possibilities for natural pest regulation in crop fields.

Such an alternative to synthetic pesticide is also acclaimed for its low persistence in the environment and low mammalian toxicity (Schmutterer, 1985; Guleria & Tiku, 2009; Isman *et al.*, 2011), hence leaving a less ecological footprint.

2.3 Challenges of Using Plant Extracts

2.3.1 Processing, Regulation and Standardization of Plant Extracts

Prominently reported challenge of using local plant extracts is a limited understanding of mechanisms and fates under natural conditions (Negi, 2012; Dougoud *et al.*, 2019). Smallholder farmers use plant extracts in less validated criteria, dosage, specification on efficacy, target pests and established risks to non-target organisms. In this case, plant extracts struggle in competition with the rapidly growing presence of improved semi-synthetic pesticides that are relatively cost-effective compared with the previous synthetic formulations. Competition lies in stringent regulatory forces that favour less natural products (Isman, 2006). On the other hand, local use of botanical products requires harvesting, processing, and frequently applying of extracts to ensure efficiency in controlling the targeted organism. Growing, collecting and processing plant extracts require heavy workload and time hence presenting a challenge for the majority of local communities who depend mainly on human labour. Cardellina (2002) highlighted maintaining and producing adequate materials and good quality herbal raw materials as main challenges in the natural products sector.

Another challenge when using plant extracts is standardization and quality control (Cardellina, 2002). Factors such as variability in plant chemistry, preparation processes such as harvesting, drying, grinding, and storing lead to variation in the ultimate chemical constitutions of the product (Soares & Ferreira, 2017) and hence compromise the quality. Processes involved in the whole plant extracts preparations under local conditions are variable and of quality that would not qualify for any registration according to the (FAO & WHO, 2017). Therefore, the competence in comparison to conventional pesticides is low, compromising commercialization at a global level. Further, plant extracts contain compounds that could also be risky to human being and other non-target organisms (Dara *et al.,* 2000) hence posing threats rather than benefits.

2.3.2 Natural Phytochemical Variation in Plant Extracts

Production of phytochemical compounds in a plant is not uniform; neither is it defined but influenced by different factors (Kang *et al.*, 2014; Verma & Shukla, 2015). Variations occur when composition and concentration of chemical compounds are produced non uniformly across plants. Several factors from within or outside the individual plants cause such variations.

Phytochemicals in plants include flavonoids, saponins, phenols, terpenoids and alkaloids, which are naturally produced by plants under specific triggering mechanisms (Bourgaud *et al.,* 2001). Production of such chemicals is known to vary such that types of chemicals and their concentrations could be different under various conditions (Verma & Shukla, 2015). Changes in the environment such as altitude (Gulzar, 2017) and development stage of a particular plant species (Zribi *et at.,* 2014) are example es of factors that influence variation. Figueiredo *et al.* (2008) reported a case of changes with the growing stage explaining that there can be an increase in the yield compounds from 10% to over 70% when a bud changes into a flower. Therefore, harvesting of plant materials requires rigorous analysis of the presence of chemical compounds to ensure the activity of the plants extracts.

Furthermore, changes in amounts of phytochemicals may occur during transportation of the synthesized chemical compounds from one organ to another. One example is the translocation of nicotine alkaloids from the roots to the leaves where they function (Yazaki, 2005). Variations may occur when sites of production encounter conditions that are different from the place of destination caused by biotic or abiotic influences (Ballaré, 2014; Koricheva & Barton, 2012; Matsuda *et al.*, 2015; Stam *et al.*, 2014).

Chemical compounds in plants may vary with time and place (Bat *et al.*, 2018; Kamanula *et al.,* 2017; Mkindi *et al.,* 2019; Patil *et al.,* 2013; Scognamiglio *et al.,* 2014). Such variation originates from changes in substrates and enzymatic reactions resulting in the production of such secondary metabolites in a plant (Tiago *et al.*, 2017). Temporal variation exists in medicinal plants where, differences in concentration of secondary metabolites may occur between summer and winter seasons (Botha *et al.,* 2018). Seasonal variations may result in changes in levels, hence resulting in fewer compounds in one season and more in another season or resulting into presence or absence of secondary metabolites depending on seasons.

A study conducted by Mbakidi-Ngouaby *et al.* (2018) showed that, of the 35 selected secondary metabolites from *Pseudotsuga menziesii* wood, lowest metabolite occurred in winter and higher concentrations in autumn.

Again, a study on essential oils showed an increase in oil production from *Plectranthus amboinicus* (Lour) during the spring season and a decrease during winter (El-Hawary *et al.,* 2013; Gouvea *et al.,* 2012). Another study conducted by Kamanula *et al.* (2017), showed that the presence of perillaldehyde and limonene occurred in higher amounts between June and August as compared with other months of the year.

2.3.3 Influence of Chemotypes on the Activity of the Pesticidal Plants

Plants can exhibit variation in chemical activity based on the chemotype variabilities. A chemotype is defined by Zribi *et al.* (2014) as "subspecies of a plant that has the same morphological characteristics (relating to form and structure) but produce different quantities of chemical components". Sources of variations in chemotype are still speculative, although Clarke (2008) suggests genetic influences. Plants species such as *T. vogelii* is known to be useful for insect pests management, and crops yield improvements (Belmain *et al.,* 2012; Mkindi *et al.,* 2017). Impressive as it is, reports show that variations in the chemotype may influence its activity and hence influence its uptake, especially for smallholder farmers (Mkindi *et al.,* 2019). Stevenson *et al.* (2012) identified Chemotype 1 and 2, and later, an additional chemotype 3 was reported by Mkindi *et al.* (2019). From the former identification, chemotype 2 was ineffective against insect pests because of the absence of rotenoids, the compounds which are known to have pesticidal properties (Belmain *et al.,* 2012). Therefore, mixing plant materials from various locations can help to attain homogenized plant extracts. However, chemical profiles of particular plants require investigation to establish effective chemotypes.

2.4 Way Forward for Facilitating the Use of Plant Extracts

2.4.1 Communication on Local Use of Plant Extracts

Communication about plant extracts use to smallholder farming communities is a vital strategy useful to mobilize farmers for adoption of the technology. Ethnobotanical studies show inventories of pesticidal plants and evidence of use by smallholder farmers (Belmain & Stevenson, 2001; Kanteh & Norman, 2015; Mwine *et al.,* 2011). Numerous papers have reported and explained mechanisms of activity in various plant species in Africa, their pesticidal compounds and evidence of their effectiveness in pest control (Mkenda *et al.*, 2015; Stevenson & Belmain, 2016; Mkindi *et al.*, 2017; Rioba & Stevenson, 2017).

Fewer studies, however, have considered field studies where smallholder farmers are also involved in obtaining information directly. For example, the ineffectiveness of plant extracts seems to have a connection with the variations in the presence and concentrations of pesticidal plant extracts and compounds (Masa *et al.*, 2016; Kamanula *et al.*, 2017; Mkindi *et al.*, 2019) which is less understood by local farmers. However, the information is essential to ensure attainment of effectiveness in the use of pesticidal plants (Moore *et al.,* 2014). Lack of evidence on the existence of variations may lead to the application of plant extracts that are less effective, hence unable to control pests and discourage users. Clear communication of differences in secondary metabolites to smallholder farming communities is thus an important aspect to ensure effective use results.

One example of communication to farmers about mitigating variations would be to mix separately collected plant materials from various locations to homogenize existing chemical compounds. This methodology was used by a Mkenda *et al.* (2015), Mkindi *et al.* (2017) and Tembo *et al.* (2018) to prepare extracts for controlling insect pests under natural conditions. Farmers would need to use plants harvested from different altitudes, landscapes, and seasons and then make a single pesticidal plant extract from the mixture. Likewise, dissemination of chemical variation results in smallholder farming communities such as the advice from this thesis may be another strategy towards effective use of pesticidal plants. Some studies have availed secondary metabolites variations among African plant species. For example, studies involving *Lippia javanica* and *T. vogelii* by Kamanula *et al.* (2017) and Mkindi *et al.* (2019) respectively were conducted in Eastern and Southern Africa to communicate strategies of helping farmers to improve their awareness on chemical variations on these plants. Mechanisms to deliver the information to farmers such as Farmer Research Networks need to catalyze dissemination of information among farmers.

2.4.2 Farmer Research Networks (FRN) for Sustainable Research Work

Facilitating farmers' participation in testing and validating technologies is a critical approach in local agricultural development. For decades, the concept of farmers' involvement in smallholder farming interventions is seen as an essential aspect known to provide representative and instrumental results and outcomes (Ashby, 1987) as opposed to conventional participation that would start with research to extension and lastly to a farmer. Ideas of farmers' involvement in agriculture emanate from the reasons that traditional research gives less chance for interactions between researchers and farmers, thereby keeping a slim opportunity for farmers to influence research objectives. As a result, researchers work on topics that may not be of the actual farmers' concerns and hence limiting uptake of obtained outcomes. The genesis of farmers' collaboration started from the extension services whereby experts reach out to the community and assist in problems through sharing required information to farmers (Anderson & Feder, 2004). From the extension service, Farmer Field Schools (FFS) encouraged participatory problem identification, on-site evaluation of existing technologies, and testing new ones (Quizon *et al.,* 2001). Apart from FFS, Holden *et al.* (2018) explained the lead-farmers approach where farmers who have more exposure and who can access the knowledge, serve as focal learning points to the rest of farmers in their communities. Lead farmers are known to influence the adoption of technologies and hence facilitating their uptakes (Fisher *et al.,* 2017). However, these approaches have been made from specific sites to give conclusions supposed to be useful in different biophysical contexts, a scenario known as "one-size-fits-all" (Nelson & Coe, 2014). Therefore, large-scale approaches need to cover a wider audience and context-specific research questions while fostering networking among farmers, stakeholders, extension services providers and researchers. A Farmer Research Network is a design under which farmers become main stakeholders from where research questions emanate and where farmers set as facilitators of the research process (Nelson *et al.,* 2019). The Farmer Research Network approach has been used in Agro-Ecological Intensification in Mali for the sorghum, maize cultivation (Huet *et al.,* 2018) and in Kenya for soil fertility and crops yield (Chebet *et al.,* 2019) as examples. Evidence from the Farmer Research Networks shows that research outputs are well understood and applied by all stakeholders (Chebet *et al.,* 2019; Nelson *et al.,* 2019), signifying a definite communication success hence it could be adopted for ensuring responsive research.

2.5 Use of Plant Extracts as Biofertilizers in Common Bean Production

The importance of using plants as alternative fertilizers arises from the need to rectify the stagnant production sustainably across much of Africa caused by several suboptimal provisioning services such as poor soil fertility and pest damage that are limiting potential yields (Bucheyeki & Mmbaga, 2013; Laizer *et al.,* 2019). A recent study presented additional evidence on the use of plant extracts for common bean crop promotion and production of essential metabolites (Mkindi *et al.*, 2020). Although fertilizers can dramatically increase bean yields, they are generally unaffordable and unavailable to most smallholder farmers as reported from Kenya (Katungi *et al.,* 2009), and contribute to reduced soil stability (Blanco-Canqui & Schlegel, 2013; Xin *et al.,* 2016), pollution (Joshi *et al.*, 2014) and carbon footprint (Hillier *et al.,* 2009).

Smallholder farmers in various parts of the world do not typically use natural fertility enhancement options successfully. For example, long time for nutrients from cover crops and mulches to be released in the soil for plant uptake as reported in Lithuania (Arlauskiene *et al.*, 2019) constrains farmers who cultivate short period crops. Likewise, Less knowledge and awareness on the use of compost as reported from Malawi (Cai *et al.*, 2019), traditional beliefs that prevent families from using livestock manure in Ethiopia (Jagisso *et al.,* 2019) and less practice of conservation agriculture in South Africa (Mtyobile *et al.*, 2019) lead to less uptake in using the available natural nutrients enhancement techniques. However, the use of plant extracts in smallholder farming systems is also an established agro-ecologically sustainable pest control method (Mkenda *et al.*, 2015; Mkindi *et al.*, 2017; Tembo *et al.,* 2018; Dougoud *et al.*, 2019; Rodríguez-González *et al.*, 2019a, 2019b). Although the economics and cost-benefits of smallholder use of crude plant extracts for pest management are certainly favourable in many situations (Mkenda *et al.*, 2015), uptake and promotion of pesticidal plants could be further facilitated by increased evidence on multiple potential benefits of their use (Rojht *et al.*, 2012) making their use even more attractive to smallholder farmers. For example, recent research has shown that the impact of plants extracts on beneficial arthropods such as pollinators and predators, is less than that observed when using synthetic pesticides (Tembo *et al.,* 2018). Research has also demonstrated that other potential benefits to smallholder use of pesticidal plants could be through direct effects on plant vigour by functioning as a green fertilizer or through the provision of additional nutrition and inducing systemic plant responses (Pretali *et al.*, 2016; Siah *et al.*, 2018).

Pesticidal plants may have additional uses such as providing fruits, seeds, fibre, timber or in traditional medicines (Isman *et al.*, 1997; Haruna *et al.*, 2013; Ngadze *et al.*, 2017). Alternative uses can also include use as green mulches and cover crops to improve the soil fertility, where previous research pointed particularly to the use of *T. vogelii* and *T. diversifolia* (Jama *et al.*, 2000; Desaeger & Rao, 2001; Nyende & Delve, 2004). Plants such as *T. vogelii* and *T.*

diversifolia are known candidates for growth improvements, yield and metabolism of common beans. Their effect on auxin transport could explain their contribution to growth, shoot growth, root development and nitrogen-fixing processes in legumes (Buer & Djordjevic, 2009; Buer *et al.*, 2010; Nagata *et al.*, 2016; Singla & Garg, 2017).

Plant extracts contain secondary metabolites that influence plant growth and resistance against harsh environments and hence maintaining vigour and yield. Examples of flavonoids in bean plants such as kaempferol, quercetin (Dinelli *et al.*, 2006; Hu *et al.*, 2006), and rutin (Gomez *et al.*, 2018) can mediate plant resistance to herbivores (Stevenson *et al.*, 1993) thus; their increased occurrence could enhance defence against antagonists. Amino acids such as phenylalanine and tryptophan are known to contribute to plant growth and metabolism such as auxin biosynthesis in the rhizosphere (Qureshi *et al.*, 2012), growth and nodulation (Hussain *et al.*, 2011). Hence, manipulations that increase such metabolites in common beans could be beneficial to provide sustainable production techniques for bean resistance to pests, growth and yield as reported for ginger (*Zingiber officinale*) (Ghasemzadeh *et al.*, 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Evaluation of Uptake of Plant Extracts Use by Smallholder Farmers

3.1.1 Study Area

The study was conducted in Mulama village, Hai district council in Kilimanjaro Region-Tanzania (Latitude 3°13′59.59” S Longitude 37°14′54” E). Selection of the area was based on increasing insect pests control challenges despite the availability of botanical pest control options and the ongoing project on Farmer Research Network to evaluate sustainable pest management using pesticidal plants. The study area is at an altitude of 1268m above mean sea level with the mean annual rainfall of about 1200 mm and a mean temperature of about 18°C. Conventional crops grown in this region are common beans, maize, banana and coffee. Traditionally, plant extracts use has been practised in these areas by a majorly elderly group of farmers. Few farmers had *T. vogelii* surrounding banana fields as a repellent to burrowing rodents commonly known as mole-rats (*Heterocephalus glaber*). Common beans and maize are widely grown to replace the deteriorating coffee plantation that exists as a cash crop. The primary constraint in common bean and maize production has been insect pests which is majorly treated by using synthetic pesticides that are costly and unhealthy. Farmers spray synthetic pesticides heavily on common beans and maize because of the high proliferation of insect pests, pests’ resistance, and extensiveness of monoculture. Information reported in this study consists of data collected based on a case study on farmers’ perceptions and experiences about using pesticidal plants in common beans fields. Individual questionnaires and focus group discussions (FGD) informed the social status related to pesticidal plant use in the study area.

3.1.2 Questionnaire Survey

A structured questionnaire was conducted to evaluate the social-economic status of farmers concerning sustainable insect pest control and participation in the use of plant extracts. The interviews involved Seventy-seven (77) participants. The consent for participation in the survey was sought before starting administering questions where the researcher explained the reason for asking questions and request for a farmer’s permission. Moreover, an approval from the Agricultural office in the district, the ward and village authorities were granted before reaching out to farmers. The study was conducted from October 2017 to January 2018. Data collection was done using a digital method through kobotoolbox built-in using the link in Appendix 7. Table 1 shows an overview of the questions included in the questionnaire survey.

Table 1: Overview of the Questions Included in the Administered Questionnaire and Focus Group Discussion

Data Group	Description
Personal data, economic profile and farms characteristics Gender; Age; Education; Household size; Yields; Land ownership Knowledge of common bean pests and weeds	Gender; Age; Education; Household composition; main occupation; Land ownership, farm landscape and distance from the household to farmland,
Using botanical insecticides	Costs, benefits, balance and the future incentives towards using botanical insecticides

3.1.3 Focus Group Discussion

The FGD consisted of nine groups that included 81 participants. Farmers signed the list of participants to ascertain their consent to participate in the discussion. The FGD took place between November and December 2017. Selection of the focus groups was based on the pre-existing groups. Participants in the FGD were engaged in the Farmer Research Network (FRN) Program to evaluate sustainable crop pest management using pesticidal plants. The FGD centred on the participatory elucidation of challenges and benefits of using plant extracts as pest management technology, the balance between challenges and benefits and the future drivers, which influence shifting from problems to benefits of using plant extracts. Groups were attended separately for an average time of two hours in each group. Notes taking and audio recording were employed during the conversations as tools for information gathering. The FGD followed a specific guideline to generate information about the challenges, benefits, and future options of the technology of using plants extracts (Fig. 1). For every theme of discussion, farmers were requested to rank the ideas based on their importance in their contexts. Participants in the FGD involved farmers who were in the program for two years, including those who dropped out as well as slow to adopt individuals. Farmer groups were formed as a result of previous pesticidal plants on-farm experiments (Mkindi *et al.*, 2017).

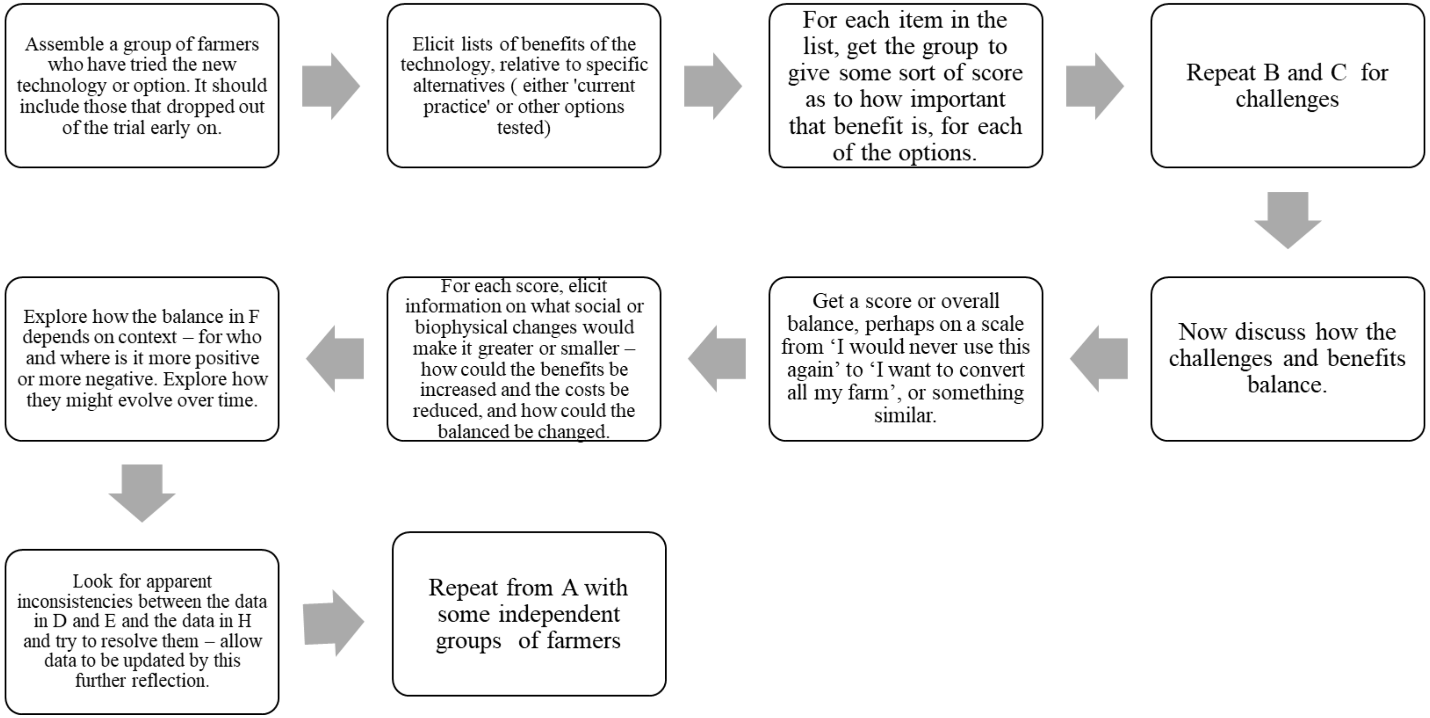


Figure 1: The Guiding Method in the Focus Group Discussion

3.1.4 Data Processing and Analysis

Demographic information from the interviews, including frequencies and percentages, were analyzed using the Statistical Package for Social Sciences (SPSS) version 20. Field visit reports, communication notes and feedback reports were documented and used as additional points during the discussion in this dissertation. Focus group information from the notebooks and audio recorder were transcribed for four days after the activity. The transcription was done along with the translation of the conversations from Swahili into the English language.

After the transcription, coding of crucial information was done by reviewing the transcript, sorting, and coding key issues under appropriate categories. Critical opinions highlighted during the FGD were coded and mapped using the NVivo qualitative data analysis software version 12 while the ranks or scores were taken as the farmers' opinions and presented in excel.

3.2 To Evaluate the Efficacy of Plant Extracts on Common Bean Insect Pest, Beneficial Arthropods and Grain Yield on Farmer’s Fields

Field observations on the farming practices related to insect pest control were evaluated in two cropping seasons in 2018 and 2019 between March and July each year. Farmers’ activities in the study area involved cultivating common beans among other crops, including maize, banana and coffee. During the cropping season, each farmer prepared experimental plots treatments with pesticidal plants and control treatments, which included either untreated plot or synthetic pesticides treatment.

Smallholder farmers were practising crop pest management using plant extracts as part of the FRN projects run by The NM-AIST in collaboration with the Natural Resources Institute - University of Greenwich. Field plots measuring 25 m^2 in the farmers’ fields were set aside for the experiment while the rest of the field was left for a farmer’s preferred farming strategy. Farmers were allowed to choose the type of pesticidal plant and the control treatment to use, as shown in Table 2.

Table 2: Summary of Pesticidal Plants, Plant Extracts, Application Frequency and Crop of Interest During Field Experimentation with Farmers

Study site	Kilimanjaro
Plant species selected	*T. vogelii*, *T. diversifolia*, *L. camara*
Crop species	Common bean (*P. vulgaris*)
Preparation of plant extracts	Extracts of 10% concentration (w/v) using 0.1% soap
Application rate	Optional between once per week, once in two weeks and application upon seeing the damage
Application method	Knapsack sprayer

3.2.1 Data Collection Process

Recording of insect abundance and damage was done every week for ten weeks. Five plants inside the 25 m^2 block were randomly selected from the centre of the plot and sampled for insect pest counting. An entire plant was inspected, and insects of interest were counted and recorded. Evaluated insect pests included aphids (*Aphis fabae* Scopoli) (Hemiptera: Aphididae), bean foliage beetle (*Ootheca mutabilis*) (Schönherr) and *O. bennigseni* Weise) (Chrysomelidae: Galerucinae), flower beetle (*Epicauta albovittata* Gestro and *E. limbatipennis* Pic) (Coleoptera: Meloidae) and pod suckers (*Clavigralla tomentosicollis* Stål, *C. schadabi* Dolling and *C. hystricodes* Stål) (Hemiptera: Coreidae).

Also, beneficial arthropods namely ladybird beetles (adults and larvae) (Coccinellidae), spiders (Araneae), lacewings (Chrysopidae) and hoverflies (Syrphidae), were recorded. Large insects were counted individually, except for aphids whose number is often high where an index of 0 = None; 1 = A few scattered individuals; 2 = A few isolated colonies; 3 = Several isolated colonies; 4 = Large isolated colonies; and 5 = Large continuous colonies; was established to assess their abundance. The severity of infestation was recoded using established categories of; 0 = No damage; 1 = Showing damage up to 25%; 2 = Damage from 26%–50%; 3 = Damage from 51%–75% and 4 = Damage more than 75%. as explained by Mkindi *et al.* (2017). Finally, the grain yield was recorded in each treatment plots by recording weight in kilogram per plot. The similar data collection process was applied both on farmers' fields as well as demonstration plots.

Analysis of variance (ANOVA) was used to analyze differences in the abundance of insects, damage and common bean grain yield. Least Significant Difference separated means at 95% confidence intervals. Analyses were performed using XLSTAT version 2019.2.2.59614, XLSTAT statistical and data analysis solution. Boston, MA, USA.

3.3 Evaluation of Spatio-temporal Variation of Production and Concentration of Chemical Compounds of *T. vogelii*

3.3.1 Samples Collection

Plant leaf samples were collected from farmers' fields. Farmers identified the specific *T. vogelii* plants that were used for controlling crop pests and diseases and for medical uses. In addition, identification of the plant was made by an experienced botanist from the Tanzania National Herbarium. In Tanzania, leaf samples of *T. vogelii* were collected over two seasons, the wet season and dry season in 14 sites located across five regions; Arusha, Kilimanjaro, Morogoro, Mbeya and Iringa. The five regions were identified after revising *T. vogelii* collections preserved in the national herbarium in Tanzania to identify possible areas where the plants could have been growing. Samples were collected in March and September 2018 during the wet and dry season, respectively. In each region, two sites were identified where samples were obtained depending on the availability of the plant at that time.

Herbarium samples were collected and assigned voucher numbers, processed and stored in the National herbarium. Rainfall data for the months of sample collection were obtained from the Tanzania Meteorological Agency (TMA), Tanzania. In Malawi, samples were collected in the Lilongwe area on farmers' fields. Likewise in Kenya, collections were made on farmers' fields in Kisumu, Homa Bay, Migori and Siaya counties, the western region; Kakamega, Busia, Bungoma, Mumias, and Central Kenyan counties. A total of 28 samples were collected in Tanzania that included 14 samples for each of the dry and wet seasons. In Malawi, 20 samples were collected from Lilongwe area between May and November 2018, while in Kenya, a total of 57 samples were collected between February and April 2019. Collected samples were dried under the shed, packed into plastic zip bags and stored in dark and dry conditions under ambient temperature before being processed and analyzed.

3.3.2 Survey of Farmers' Awareness on the Use of *T. vogelii*

Twenty-two farmers from six Tanzanian regions were interviewed in the survey to determine the uses of *T. vogelii* in the household. The survey was done using the short survey constructed in the kobotoolbox available on a link in Appendix 8. To identify *T. vogelii* uses with reference to the type of sample collected, only farmers who owned the plant or were neighbours to the farmer owning the plants were interviewed. The selection of farmers, therefore, did not follow specific social survey protocols for sample sizes selection.

3.3.3 Sample Analysis

Dried *T. vogelii* samples were powdered using an electric grinder (SALTER, Model No EK2311ROFB distributed by UP Global Sourcing, Victoria Street, Manchester, OL9 0DD, UK Made in China). Tephrosia powder (50 mg/mL) was extracted in methanol. Each extract was left to stand for 24 h at room temperature before chemical analysis. Plant leaf extracts were transferred to Eppendorf tubes and centrifuged for 20 min at 5000 rpm. The supernatant (300 µL) was transferred into HPLC vials for analysis. Extracts were analyzed by liquid chromatography-Electrospray Ionization Mass Spectroscopy (LC-ESIMS) and Ultraviolet (UV) spectroscopy using a Thermo Fisher Velos Pro LC-MS. Samples (5 µL) were injected directly on to a Phenomenex Luna C18 (2) column (150 Å~3 mm i.d., 3 µm particle size) at 400 µL min^{-1} and eluted using a linear gradient using water (A), methanol (B) and 1% formic acid in acetonitrile (C) where rations of A:B:C were 90:0:10 at t = 0 min to 0:90:10 at t = 20–25 min), returning to 90:0:10 at t = 27–30 min. The column was maintained at 30ºC. Compounds were detected on a Thermo Fisher Velos Pro Dual-Pressure Linear Ion Trap Mass Spectrometer. Samples were scanned, using Fourier-Transform Mass Spectrometry (FTMS), from m/z 200–600 corresponding to the range of molecular ions expected in samples of *T. vogelii*. Ultraviolet (UV) peak areas were quantified against a calibration curve of an authentic in-house standard (Stevenson *et al.,* 2012). The resulting peak areas of deguelin were measured at a wavelength of 300 nm and arranged in an excel file for statistical analysis. Presentation of data and sampling points, graphical presentation of chemotype and variation in amounts of chemotype 1 in *T. vogelii* was performed using Aeronautical Reconnaissance Coverage Geographic Information System (ArcGIS), version 10.3. 3.3.

3.3.4 Statistical Analysis

Analysis of Variance and descriptive statistics, proportion analysis and regression analysis were performed using XLSTAT version 2019.2.2.59614, XLSTAT statistical and data analysis solution. Boston, MA, USA.

3.4 Evaluation of the Contribution of the Application of Plant Extracts on Bean Plant Growth Promotion and Grain Yield Increase

3.4.1 Bean Planting and Plant Materials Preparation

The experiment was carried out in a controlled pest-free glasshouse at the NM-AIST (Latitude 3º24′S Longitude 36◦47′E, the elevation of 1168 m above mean sea level with a mean annual rainfall of 1200 mm, mean maximum temperature of 21.7ºC and mean minimum temperature of 13.6ºC). Each treatment unit consisted of eight bean plants. Common bean seeds used for the experiment were of Lyamungo 90 variety, purchased from the Tanzania Agriculture Research Institute (TARI)-Selian center. Two seeds were planted in each pot, later thinned to one plant per each pot, using 2-litre volume pots containing standard potting compost. All pots were arranged in a randomized complete block design on a bench in the glasshouse, providing even lighting, ventilation, temperature (25±5ºC) and equal amounts of water per pot.

Pesticidal plant materials (*T. vogelii* and *T. diversifolia*) were collected from Lyamungo field areas, dried under the shade and ground into a fine powder using previously reported methods (Tembo *et al.,* 2018). Plant species namely *T. vogelii* and *T. diversifolia* are among a large group of insecticidal plants that have been used for decades for pest control (Grzywacz *et al.*, 2014; Mkenda *et al.*, 2015; Mkindi *et al.*, 2017; Tembo *et al.*, 2018). Positive controls included synthetic pesticide (Karate, lambda-cyhalothrin) and a commercial foliar fertilizer (Bio-Force, an organic extract from seaweeds and blue-green algae) which were applied according to instructions provided on the respective labels.

Pesticidal plant powders were extracted in soapy water (0.1% soap) to produce an extract solution of 10% (w/v) following previously reported methods (Tembo *et al.,* 2018). Negative control treatments were with plain water and water with 0.1% soap.

Either all treatments were applied in two different methods, as a foliar spray using a hand sprayer or directly to the soil with a small watering can, ensuring equal amounts were applied to each plant. The treatments were applied fortnightly from the first week after plant germination until the time of bean flowering, i.e., a total of four treatment applications.

3.4.2 Collection of Growth Parameters Data and Leaf Samples for Chemical Analysis

Growth parameters and samples for chlorophyll content and bean leaf chemistry analysis were collected before bean flowering. Yield parameters were collected close to the maturity of the beans and the grain yield collected after final bean harvesting. The growth parameters that were measured included plant height, number of leaves, number of branches, main stem width, leaf area and leaf greenness. Leaf greenness was scored using a scale of 1-5 where 1 was regarded as low greenness and 5 as high greenness using a leaf colour chart as previously reported (Haripriya *et al.*, 2008). Leaf area was determined from the direct measurements of the length as a distance between the base and apex of the leaflet, and the width between positions of the leaflets. Leaf area was then calculated using the formula described by Bhatt and Chanda (2003) as follows;

$$LA = 11.98 + 0.06\ L \times W \tag{1}$$

Where: LA = Leaf area , L = Leaf length and W = Leaf width

Plant leaf samples were harvested three days after spraying beans. Harvesting was done at the vegetative stage, just before flowering. Four plants from each treatment were randomly selected from each plant. The leaves were thoroughly washed with distilled water. Two leaves from each plant were placed in a desiccator with silica gel, desiccated and prepared for phytochemical analysis. The other two leaves collected from each plant were used for spectrophotometric analysis described below.

(i) Chlorophyll Content Analysis

Chlorophyll concentration was determined through the extraction of chlorophyll from the third leaf of the growing tip of each plant using Dimethyl Sulphoxide (DMSO) as described by Hiscox & Israelstam (1980). This involved placing 100 mg of the middle portion of the leaf in a 15 ml vial containing 7 ml DMSO and incubating at 65ºC for 24 hours after which the leaves were completely transparent signifying chlorophyll extraction. The extracted liquid was transferred to graduated tubes and made up to a total volume of 10 ml with DMSO and then kept at 4 °C waiting for analysis.

To determine the chlorophyll content, 300 microliters of the sample were transferred into an 86 well plate, where the absorbance at 645 nm and 663 nm were read using a spectrophotometer (Synergy, Multi-mode reader, Biotek Instrument Inc. USA) against DMSO as a blank. Chlorophyll levels in milligrams per litre (mg/l) were then calculated using the formula described by Arnon (1949) as follows;

Total Chl = 20.2 x D645 nm + 8.02 x D663 nm (2)

Where: Chl = Chlorophyll and D = Absorbance value at the respective wavelengths obtained from the spectrophotometer.

(ii) Anthocyanins and Flavonoids Analysis

Flavonoids and anthocyanins in bean plant leaves were determined using the method described by Makoi *et al.* (2010). Dried and ground bean leaves were used, where 0.1 g of the plant powder was extracted in 10 ml acidified methanol, made at a ratio of 79:20:1 of MeOH: H_2O: HCl. The extract was incubated for 72 hours in darkness for auto extraction and then filtered through a filter paper (Whatman #2). The absorbance of the clear supernatant was measured at 300, 530 and 657 nm in a spectrophotometer (Synergy, Multi-mode reader, Biotek Instrument Inc. USA) against acidified methanol as a standard. Flavonoid concentration was obtained from the measured absorption at 300 nm and expressed in Abs g DM^{-1} whereby

Abs g-1 DM = Abs 300 (3)

Anthocyanins were measured as using the formula described by Lindoo and Caldwell (1978)

Abs g-1 DM = Abs 530 - 1/3 x Abs 657 (4)

Where Abs = Absorption readings recorded from the spectrophotometer. The resulting concentration was expressed as Abs g DM^{-1}.

3.4.3 High-Performance Liquid Chromatography (HPLC) Detection of Primary and Secondary Metabolites

Desiccated bean leaves were powdered using an electric coffee grinder, and 50 mg of the powder was extracted in methanol (1 ml) and left to stand for 24 hours at room temperature before chemical analysis. Extracts were transferred to Eppendorf tubes and centrifuged for 20 minutes at 500 rpm. From this 300 µl of the supernatant was transferred into HPLC glass vials for separation. The sample analyses were performed by Liquid Chromatography-

Electrospray Ionization Mass Spectroscopy (LC-ESIMS) and UV spectroscopy using a Thermo Fisher Velos Pro LC-MS. Aliquots of extract were injected directly onto a Phenomenex (Macclesfield, Cheshire, United Kingdom) Luna C18(2) columns (150 × 3.0 mm i.d., 5 um particle size) and compounds eluted using methanol (A), water (B) and acetonitrile containing 1% formic acid (C) with A = 0%, B = 90% at T = 0 min; A = 90%, B = 0% at T = 20 min and held for 10 min with C at 10% throughout the analyses. The column temperature was 30ºC with flow rate = 0.5 ml min^{-1}. High-resolution MS spectra were used to provide additional data for compound identification and were recorded for a subset of samples using a Thermo LTQ-Orbitrap XL mass spectrometer (Waltham, MA, United States) with compound separation on an Accela LC system.

3.4.4 Statistical Analysis

The experiment was conducted following the randomised complete block design with eight replications to determine grain yield and growth of common beans and four replications to determine the metabolites. Effects of treatments and their interactions observed were subjected to Analysis of Variance. The means of treatments and interactions were compared using the least significant difference (LSD) test at a significant level of $P \leq 0.05$. Principal Component Analysis (PCA) was performed to explain potential covariance between bean plant growth, grain yield parameters and common bean metabolites. All the analyses were done using XLSTAT version 2019.2.2.59614, XLSTAT statistical and data analysis solution. Boston, USA.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Social and Economic Status, Pests and Plant Extracts Use Among Smallholder Farmers

The study included beneficiaries of the FRN project to evaluate sustainable agroecological crop pest management using pesticidal plants (FRN4PP). Participants engaged in the study included (58%, n=40) female and (41%, n=28) male where most of them were in the age range of 50 and above (58.8% n=40). Family sizes of farmers were evaluated using the number of household members whereby the majority of households (92.6%, n=63) consisted of up to five family members. A high percentage of farmers were literate, where the dominant level of education was primary education (91.2%, n=62). Most farmers practised intercropping (76.5%, n=68) compared with the mono-crop farming system (23.5%, n=16) and cultivated beans on rented fields (61.8%) (Table 3). Rented fields were open enough to favour bean cultivation as opposed to family farms where coffee and banana crops were mainly grown.

Table 3: Demographic and Selected Characteristics of Farmers Involved in the FRN Study

Respondent variable	Frequency (n)	Per cent (%)
Education level		
Did not attend school	2	2.9
Primary education	62	91.2
Secondary education	3	4.4
Higher education	1	1.5
Age		
20-30	7	10.3
31-40	3	4.4
41-50	18	26.5
>50	40	58.8
Family size		
1-5	63	92.6
6-10	5	7.4
Gender		
Male	28	41.2
Female	40	58.8
Land ownership		
Family farm	25	36.8
Bought	1	1.5
Rented	42	61.8
Cropping system		
Monocrop	16	23.5
Intercrop	52	76.5

(i) Pests Prevalence and Pest Control Status Among Farmers

Participants in the study reported the presence of pest incidences, where the majority (99%, n=67) acknowledged it as a tremendous challenge in common beans production. Results show that the most reported bean pest was foliage beetles (75%, n=51), followed by aphids (63%, n=43), pod sucker (11%, n=8) and flower beetle (26%, n=18). As a response to pest challenges, farmers reported using mainly two strategies, that is plant extracts and synthetic pesticides.

Using synthetic pesticides was reported less (35%, n= 24) than using the plant extracts (39.7%, n= 27). Fewer participants using synthetic pesticides was related to the results that the majority of participants (50%) were of the age of above 50 years preferred using plant extracts compared to other age groups. A few farmers (27%, n=17) reported receiving the extension services while the rest reported that there was no extension service, especially on pest management because the extension worker was a livestock officer by profession (Table 4).

Table 4: Pest Prevalence and Pest Control Strategies Among Smallholder Farmers

Variables	Frequency (n)	Percentage (%)
Pests presence		
Aphids	43	63.2
Foliage beetle	51	75.0
Flower beetle	18	26.5
Pod sucker	8	11.8
Extension services		
Yes	17	25
No	52	75
Using botanicals before the project		
Yes	27	39.7
No	41	60.3
Using synthetic pesticides before the project		
Yes	24	35.3
No	44	64.7

(ii) Age as an Influencing Factor to Adoption and Use of Plant Extracts Among Smallholder Farmers

Figure 2 below describes the trends of using plant extracts with respect to the age of participants. From the results, 50% of participants using plant extracts were elderly in the age above 50 years, followed by participants of the age ranging from 41-50 whose participation was 33.3% (x^2 = 3.94; df = 3; p = 0.0.115). Surprisingly, no one in the age range of 31-40 used plant extracts among the participants. In the age range of 41-50, the percentage of participants using plant extracts was 33.3% and (14.3%) at the age range of 21-30, hence recording a decreasing trend with the age of farmers. On the other hand, the use of synthetic pesticides was higher for lower age compared with the age above 50years (27%) although participants in the ages below 50 were less in number.

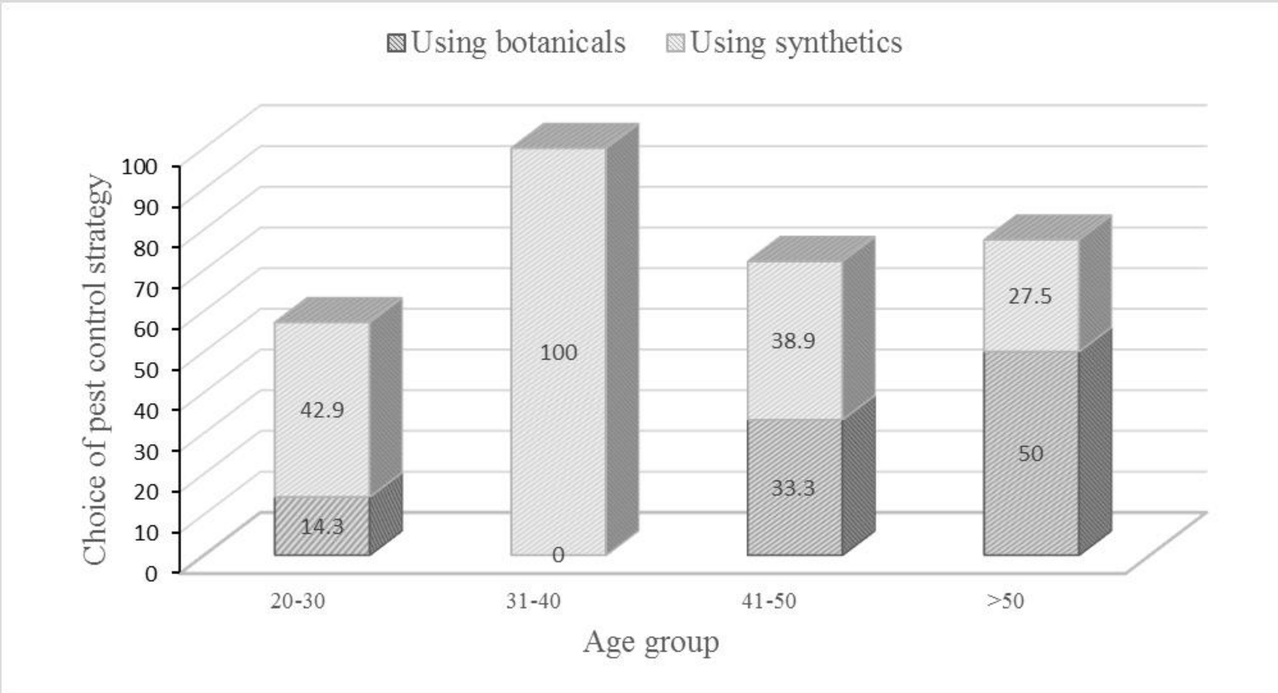


Figure 2: Influence of Age on the Preference of Pesticides Types

(iii) Benefits of Using Plant Extracts

Response to the benefits of using plant extracts from the focus group discussion varied depending on the participants' experiences on agricultural pests' control. The benefits of using plant extracts were compared with other pest management practices in the community or any other tested technology. The primary comparison was the use of synthetic pesticides, as the major pest control strategy in the area that has existed for decades. Opinions on the benefits of using plant extracts from the FGD are shown in Fig. 3. From the listed benefits, participants had a chance of discussing each benefit and gave a score based on the importance of the benefit relative to their own experience. Results in Fig. 4 shows the benefits of using plant extracts as listed by farmers showing the priorities based on their context. Benefits in this study imply the gains and the importance obtained from the technology. The benefits included experiences gained through participating in the project, and personal understanding of pesticidal plants uses.

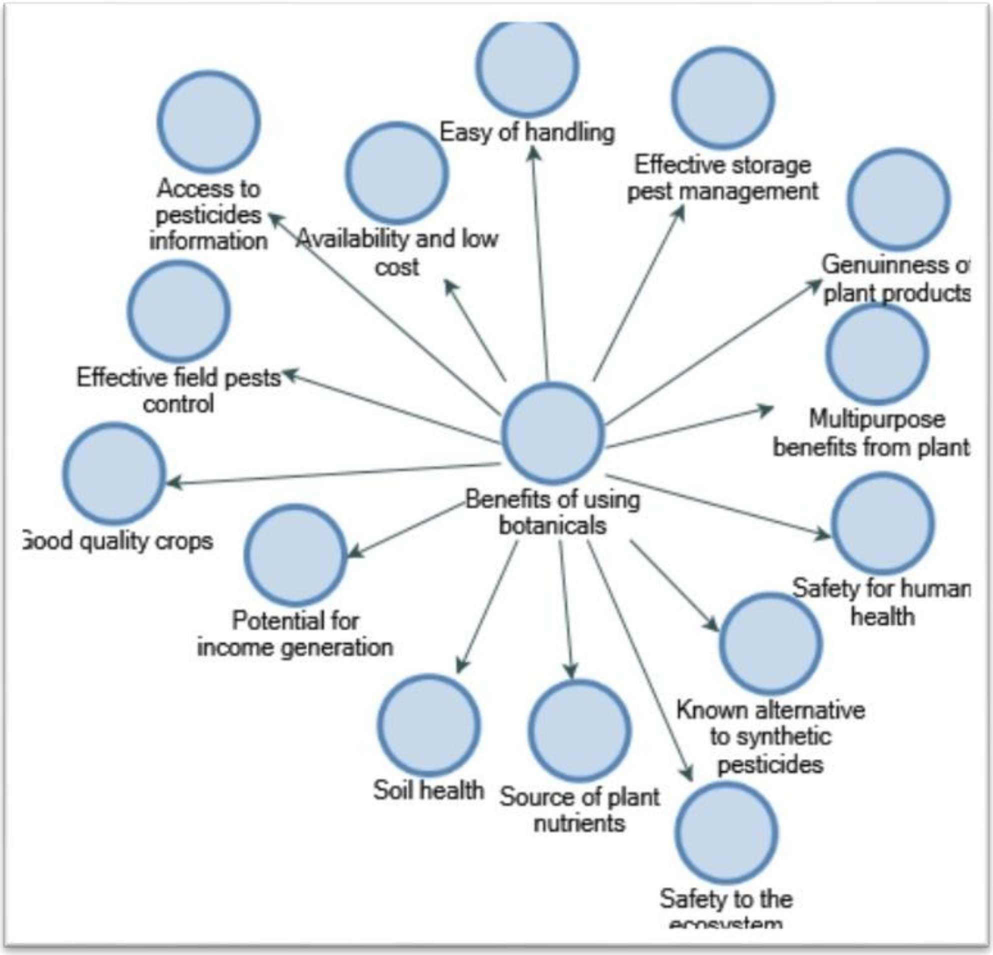


Figure 3: Coded Opinions About the Benefits of Using Plant Extracts

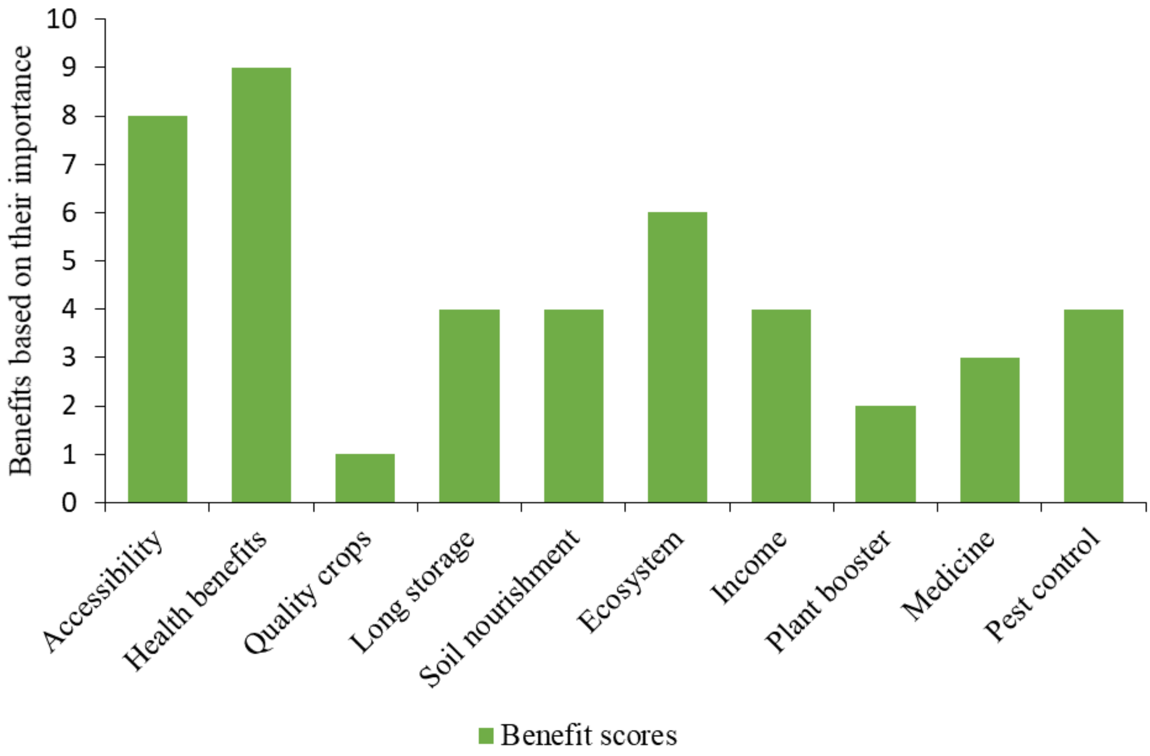


Figure 4: Ranks of Benefits for Using Plant Extracts

Ranking benefits of using plant extracts (Fig. 4) showed that the most mentioned benefit was health benefits. Pesticidal plants were perceived as less harmful to human beings' health. The second prioritized benefit was the accessibility where participants reported availability of plant materials along the field margins, roadsides and in the abandoned and uncultivated fields. Benefits to the ecosystem and the ability of extracts to control pests were the frequently mentioned benefits. The least mentioned benefits were the contribution of plant extracts to the production of quality crops and their use as a plant growth promoter.

(iv) Challenges of Using Plant Extracts

Challenges in this study were regarded as the factors that inhibited the use of plant extracts, including the pains, hardships and inconveniences that would result in less uptake of the technology. Figure 5 below highlights the challenges of using plant extracts. Like benefits, challenges were compared with any more comfortable and more feasible pest control strategy that is used among the community members or imagined to be useful. Likewise, challenges were ranked based on the level of importance.

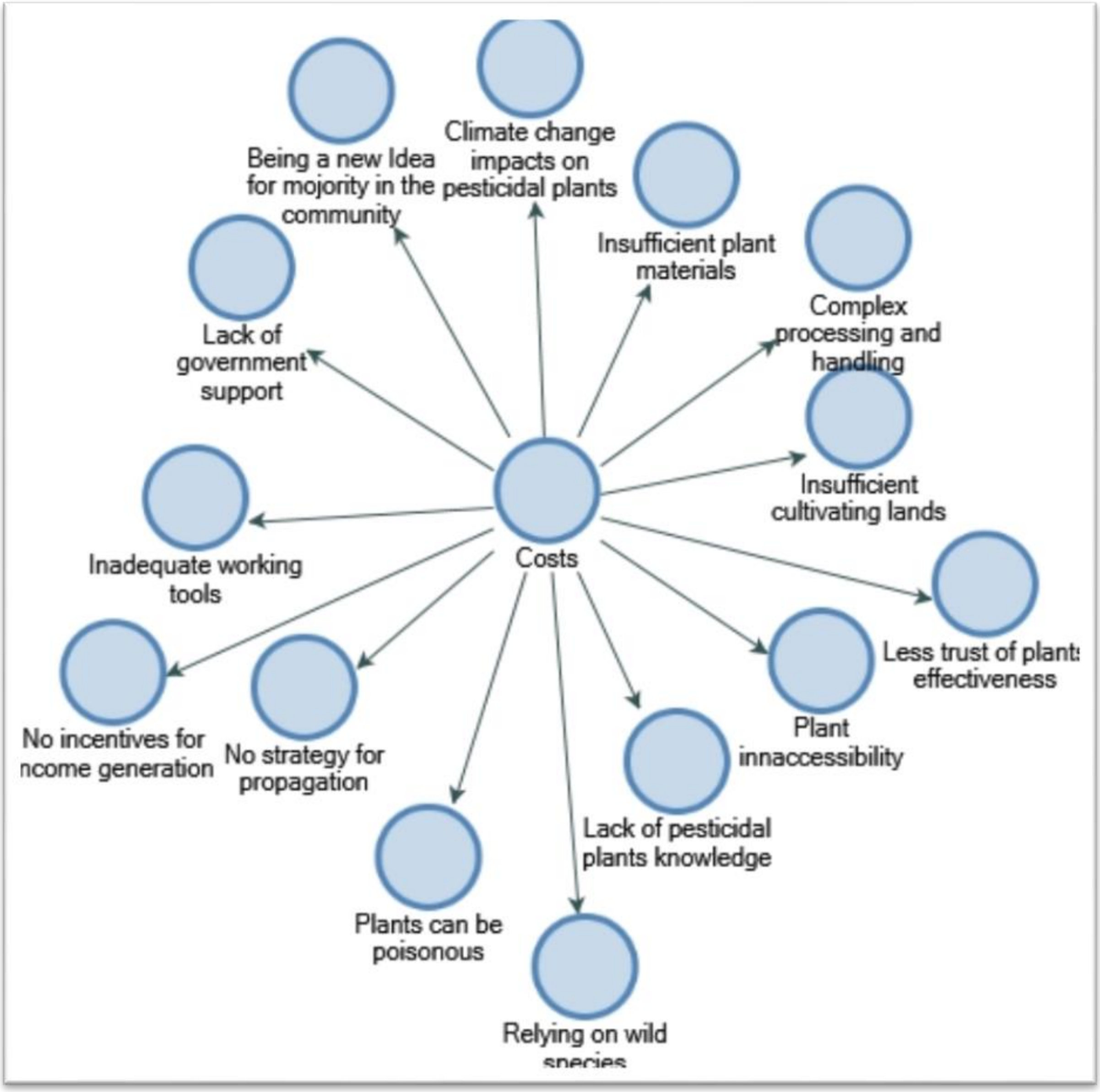


Figure 5: Coded Opinions About the Challenges of Using Plant Extracts

Likewise, challenges were scored based on their importance (Fig. 6), highlighting ranks of challenges across groups of participants. The most important or highly ranked challenges were those whose presence prevented the adoption of the plant extracts use. Tools which implied the working equipment for harvesting, processing and application of plant extracts were ranked as the most critical challenge. Mentioned tools were such as masks, gloves, grinders, filtering cloths and drying places. The second-ranked challenge of using plant extracts was the preparation process, followed by less awareness and availability of plant materials.

Least considered challenges were such as drought and the fact that plants could be poisonous. Other challenges included less government support, less trust in the effectiveness of the plant materials and the short storage time or shelf life.

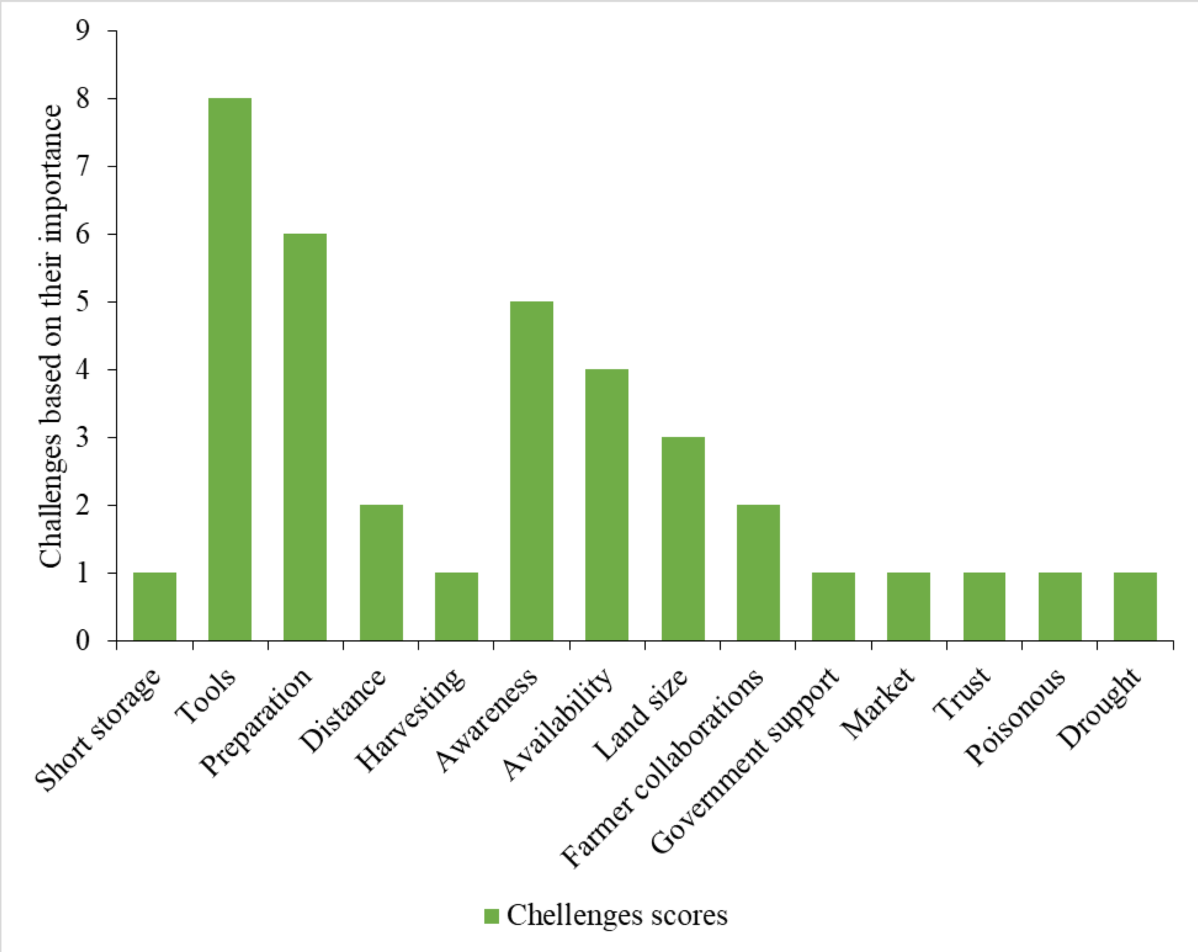


Figure 6: Scores of Challenges of Using Plant Extracts

(v) Prospects of Using Plant Extracts

Future options to enable the use of the plant extracts technology is shown in Fig. 7. Farmers were requested to highlight opinions that would assist in increasing their ability and motivation to use plant extracts. Therefore, the mentioned opinions were the required future steps which could be addressed to reduce challenges and increase the benefits.

Figure 8 shows that the most important future interventions were plants domestication, availability of tools for processing of the plant extracts and education about using them.

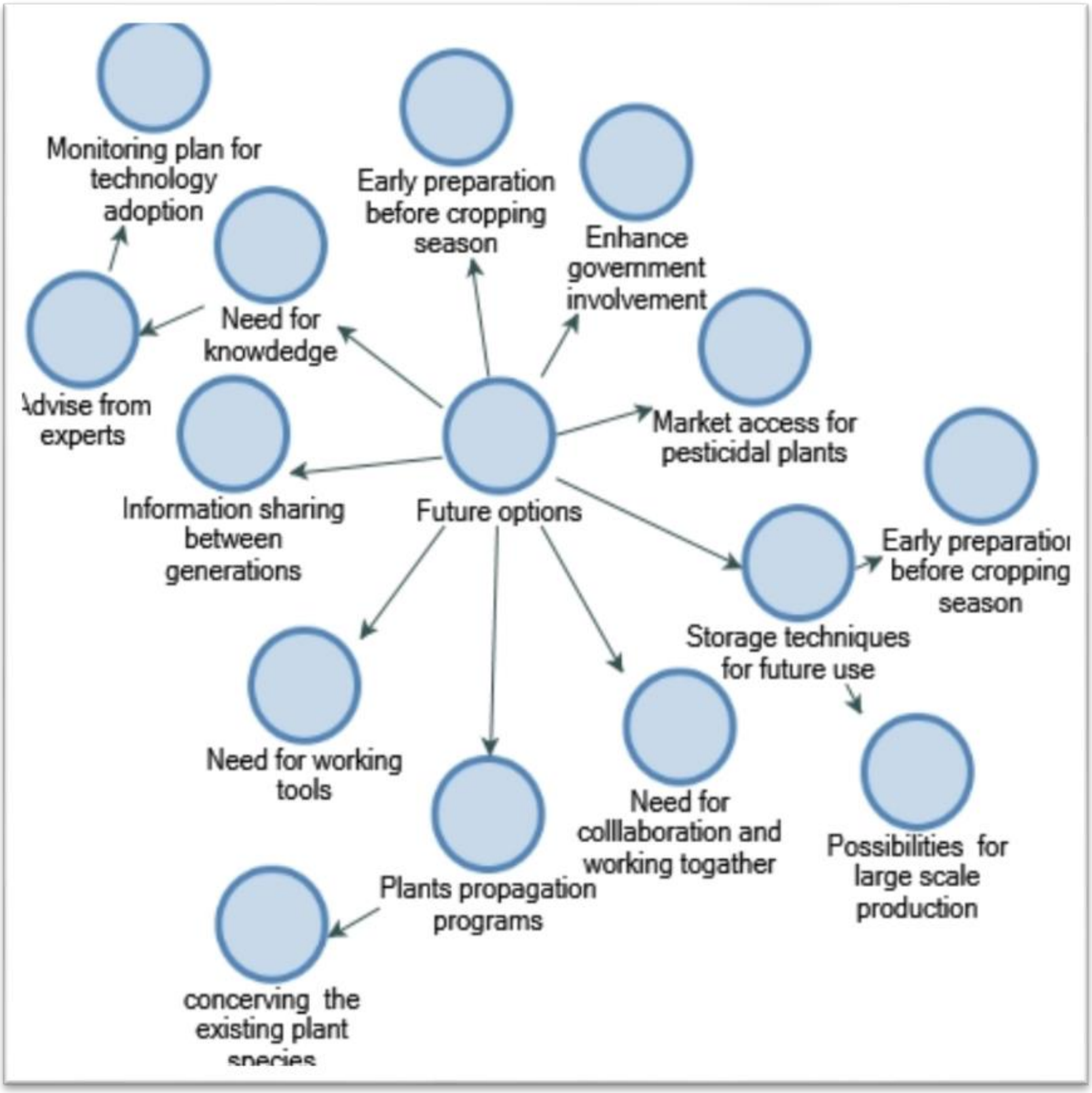


Figure 7: Mapped Options to Facilitate the Use of Plant Extracts

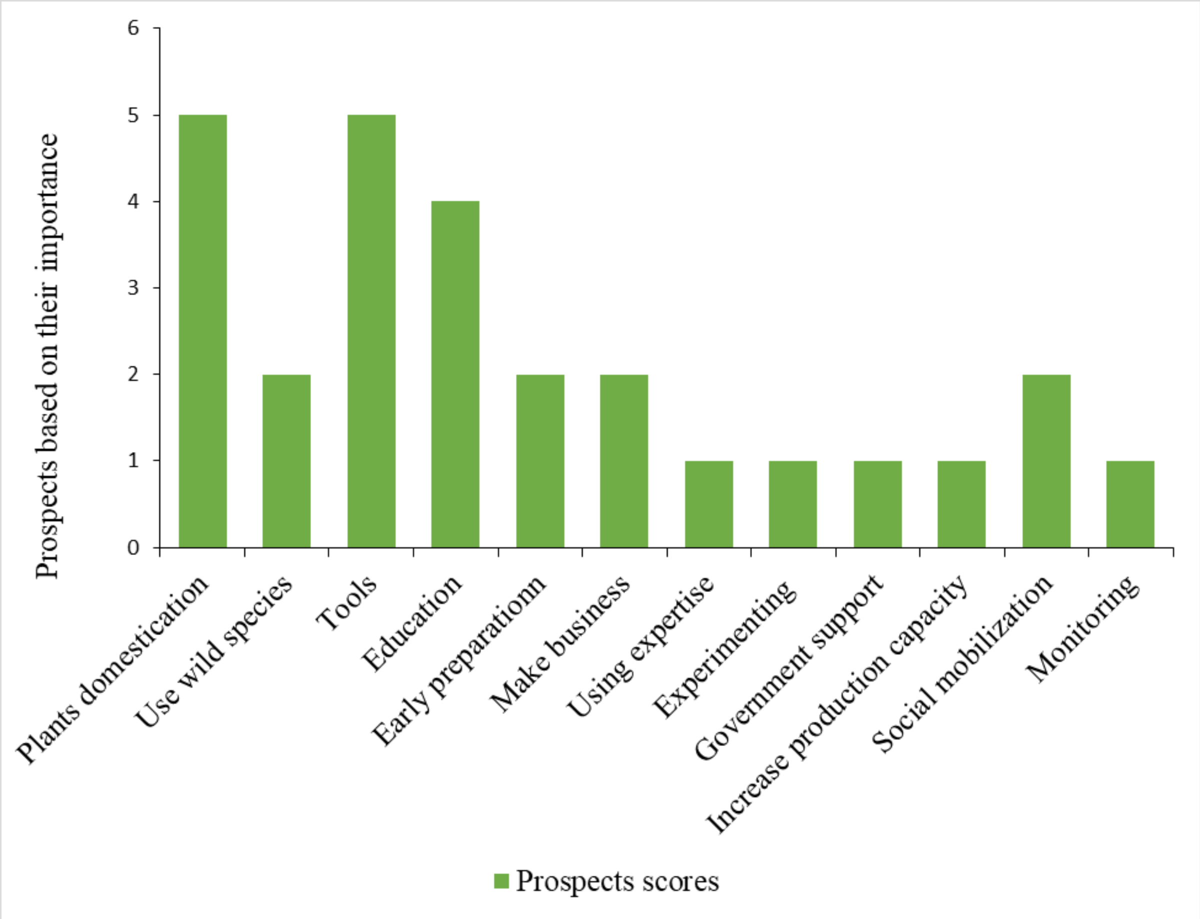


Figure 8: Scores of Prospects towards Using Plant Extracts

Using wild species, early preparations of pesticidal plants materials before the cropping season, making the plant extract use a business opportunity and social mobilization were also recorded as prospects.

(vi) Farmer's Elaboration of Benefits of using Plant Extracts

Farmers reported plant extracts to be less harmful to human beings in comparison with industrial pesticides. Plant species namely *T. diversifolia*, *L. camara* and *T. vogelii* were mentioned to have no disorders related to chest pain, breast cancer, diarrhoea, skin rashes and stomach pains on human bodies when a farmer got into contact with them, unlike the synthetic pesticides. The mentioned disorders were perceived to be associated with poisons which may come from chemicals. One participant in group 2 reported that *"Plants do not affect the chest, so you do not have to drink milk after getting into contact with it compared with when you spray synthetic pesticides."*

On a positive note, farmers reported the use of *T. diversifolia* to treat bile dysfunction in cows, coccidiosis in chicken, stomach pains, and typhoid for human beings "*To cows Tithonia help to treat bile, and in chicken to treat many diseases including kideri (coccidiosis).* Others regarded plants as harmless based on their nature; *"They don't have effects because they are natural"*. Therefore, extracts were regarded as having multiple benefits, such as human and animal health and naturally harmless.

Plant availability was another mentioned benefit of using plant extracts. Farmers reported that it was easy to access pesticidal plants. The study area is located on the slopes of mountain Kilimanjaro where the diversity of plants species is still apparent. The study area contains pesticidal plant species in various locations such as along roads, field margins, abandoned fields and in uncultivated areas. Likewise, some pesticidal plant species such as *T. vogelii* was being grown by farmers.

Participants reported that pesticidal plants were useful as a source of plants nutrients. One participant highlighted her experience of pesticidal plants on her garden; *"When I used the plant materials, plants (beans) were greener".* Greenness, as indicated by a farmer, was related to plant health.

(vii) Farmer's Elaboration of Challenges of Using Plant Extracts

Processing plant extracts was reported as among the challenges. Tools for processing plant materials, complex processes in the preparation and handling of plant extracts were highlighted as pains by the majority of groups. One participant reported; *"There is the long time involved from going to collect plant materials, drying, grinding, soaking for a day and then filtering process*" and another one added; *"This is different from the synthetic pesticides where you just go to the shops and buy a ready-made bottle of pesticides or a pack of dust material".*

Working tools such as grinding machines, drying facilities, filtering tools, large volume tanks for extracts preparation and protective equipment were the most mentioned tools. Farmers stipulated that manual preparation of plants extract was time-consuming and less effective. When grinding manually, much of the plant powder was blown by the wind, given the fact that grinding requires to be done outdoors to allow sufficient airflow. The manual pounding was reported inefficient because not all fibres would be ground into powder form. Complete grinding of plant materials into finer powder increases chances for efficient extraction when in contact with a solvent. Manual grinding was mentioned to cause respiratory complications because of the dust from plant powders.

Drying facilities and drying conditions were underlined as essential factors complicating preparation of plant extracts. Farmers dried plant materials on the house ceilings, temporary roofed stages in open spaces and indoor open spaces. Challenges reported during drying were direct sunlight which is known to break down the thermal labile chemical compounds intended for pest control. Likewise, the humid condition was identified as a source of mould growth hence affecting the effectiveness of the pesticidal plants' extracts by altering the pesticidal plant chemistry.

Low awareness about the use of plants extracts was a prominent general challenge which was related to types of information offered by agricultural experts in respective areas. Participants expressed a need for a formal version of knowledge on plants extracts use that experts would use to inform farmers.

Despite the traditional knowledge about the use of pesticidal plants, another challenge noted from the study was lack of awareness on how to process pesticidal plants because existed indigenous knowledge never informed a uniform preparation process that was proven as effective. On the other hand, traditional knowledge transfer was reported to diminish due to less formal awareness of the plant extracts use and due to the presence of alternatives such as using synthetic pesticides. From the study, youths were less aware of how to use plant extracts neither the benefits of using them. Younger people opted for easy and fast options such as buying ready-made products instead of using their efforts to invent means of modernizing use of pesticidal plants. *"At least older people can do that, but the younger generation always goes for easy things such as buying synthetics. This is manifesting in Rombo (Kilimanjaro region) where the majority of older people don't use synthetics."*

Another challenge was the lack of a designated market for pesticidal plants raw materials and products. One farmer insisted that for any technology, creating a means to earn income was a better strategy for increasing its success. He said; *"If we don't have a market we can even cut them down and leave the idea because why are shops selling chemicals and we can't sell plant extracts?"*

Participants stressed that stimulants for any innovation were the presence of an income generation avenue, and it was a means through which the idea would penetrate widely to communities.

(viii) Farmer's Elaboration of Prospects of Using Plant Extracts

From this study, farmers showed the need for education, training and appropriate tools as essential factors that would increase levels and speeds of adopting the use of plant extracts for insect pests' control. Interaction with farmers during this study showed good participation of farmers during meetings, training and field exchange activities indicating their willingness to learn. A critical future demand was education. Education in this study implied the need for practical and theoretical knowledge shared through training, experimentation and farmer-to-farmer knowledge exchange. Existence of the FRN program was a real example of education, farmer-to-farmer learning and knowledge exchange which needed to expand to the majority of farmers.

Participants reported tools and methodologies as other vital points for future investments. Presence of tools and methods was envisaged to save time and energy and increase speed and efficiency in processing pesticidal plants to obtain desired products in enough amounts. Additionally, a number of prospects such as; (a) the need to establish critical pesticidal plants in their homesteads and field margins to increase the availability, (b)seek government support and regulatory assistance towards processing and commercialization of pesticidal plants, (c) early preparation of pesticidal plants powders to shorten preparation time during cropping seasons, (d) design a business strategy for pesticidal plants and (e) receive timely monitoring from research and experts were the reported ways forward.*"We need to prepare the pesticides well in advance because if we don't, it is hard to do that during the cropping season, as you know the farming activities will be many"*.

4.1.2 Influence of Treatments on the Abundance of Pests, Beneficial Arthropods, Damage and Grain Yield of *P. vulgaris*

Evaluation of abundance of key pest, beneficial arthropod and grain yield of common beans was conducted over two cropping seasons of 2018 and 2019 from March to August each year. Tested pesticidal plants were *T. vogelii*, *T. diversifolia* and *L. camara*. These plant species are readily available in the study area and were agreed upon with farmers.

(i) Effects of Plants Extracts on the Abundance of Beneficial Arthropods

In the 2018 cropping season, higher arthropods abundance on individual farmers' plots was recorded on the untreated (Control-u) plots where ladybird beetles were significantly higher (ANOVA $F = 7.2$, $df = 14$, $p = 0.0001$) on untreated plot compared with the rest of treatments. On the other hand, the abundance of spiders (ANOVA $F = 1.3$, $df = 113$, $p = 0.3$), hoverflies (ANOVA $F = 0.8$, $df = 113$, $p = 0.5$) and lacewings (ANOVA $F = 0.6$, $df = 113$, $p = 0.6$) showed no significantly different among treatments (Fig. 9).

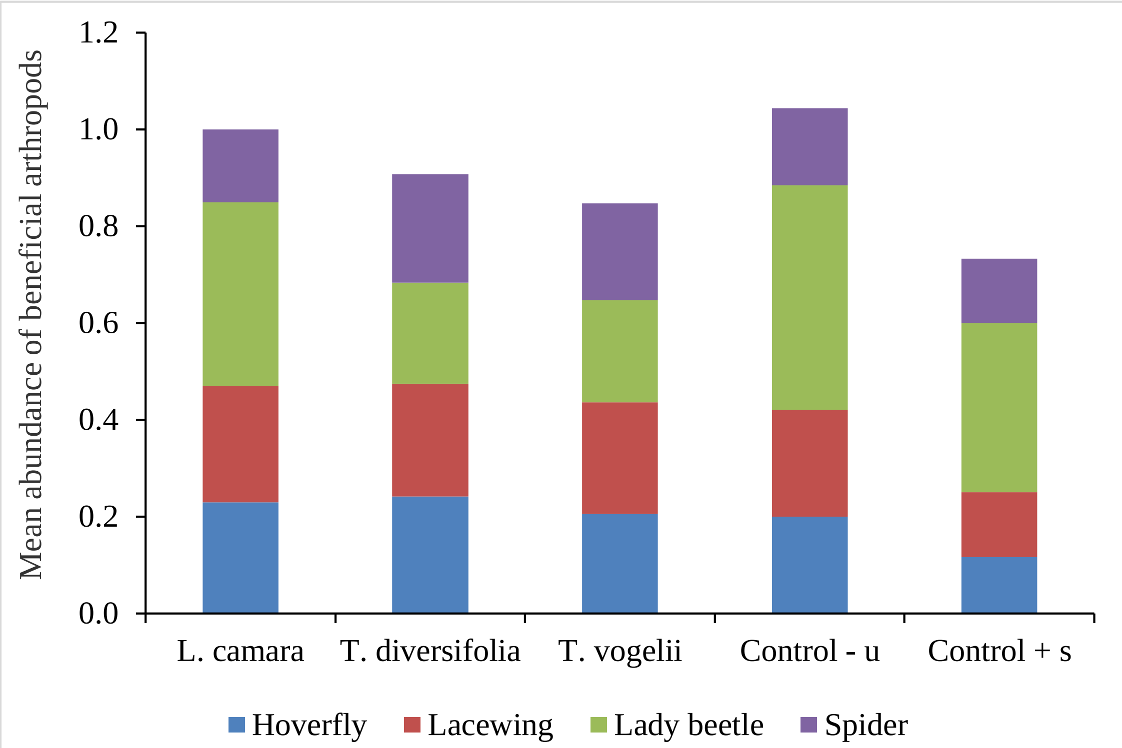


Figure 9: Beneficial Arthropods Abundance in Common Beans Individual Farmers' Plots in 2018 Cropping Season

Results in Fig. 10 shows the abundance of beneficial arthropods on the demonstration plots in the 2018 cropping season. There was no significant difference in the abundance of spiders (ANOVA $F = 1.6$, $df = 14$, $p = 0.2$), and hoverflies (ANOVA $F = 2.6$, $df = 14$, $p = 0.8$), across the treatments. However, a higher abundance of ladybird beetles (ANOVA $F = 5$, $df = 14$, $p = 0.01$), was recorded on the untreated plots and was significantly higher than *T. vogelii* and *T. diversifolia*. No significant difference in ladybird beetles was observed between untreated plots with synthetic pesticide and *L. camara*.

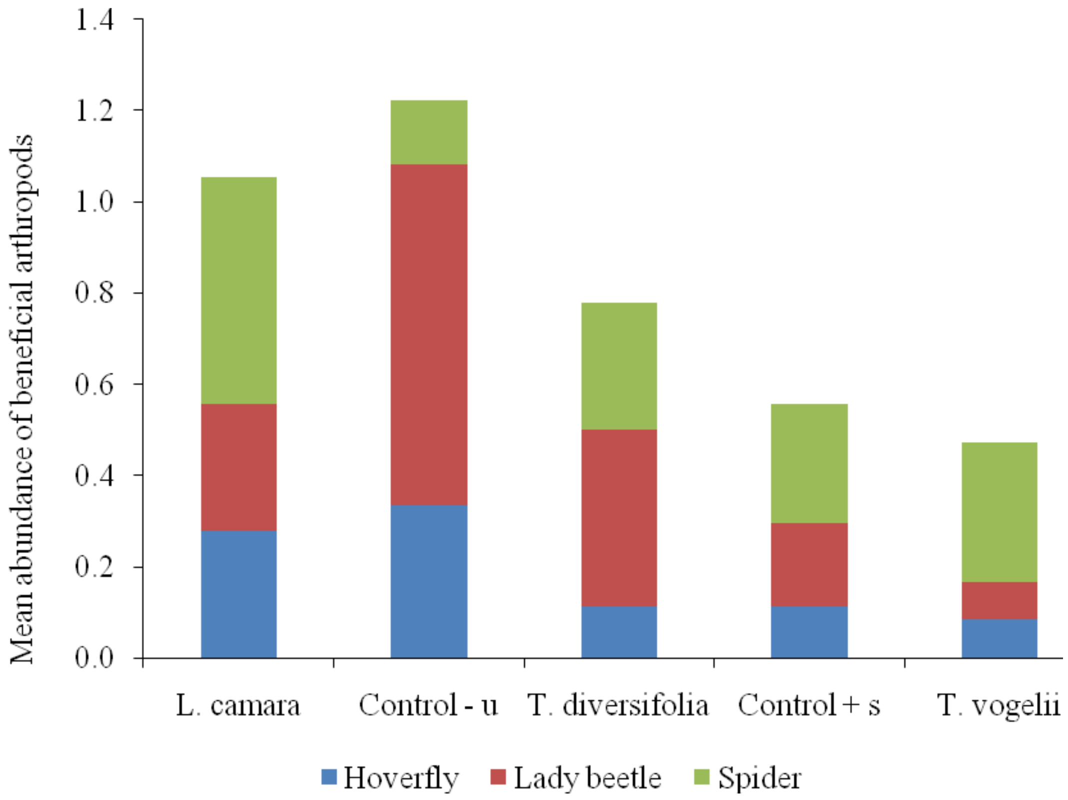


Figure 10: Beneficial Arthropods Abundance in Common Beans Demonstration Plots in the 2018 Cropping Season

Table 5 below shows the abundance of beneficial arthropods for the 2019 cropping season. A significant variation in the abundance of beneficial arthropods was observed for spiders ($P<0.05$), hoverflies (larvae) (*P<0.001*) and robber flies (*P<0.01*). Expectedly, higher abundance was found on the untreated plots (Control-u) (0.38 ±0.04, 0.39±0.07, 0.16±0.02, 0.11± 0.02, 0.45± 0.05, 0.45± 0.05 and 0.38±0.04) for spiders, adult ladybird beetles, adult hoverfly, ladybird beetle larvae, adult lacewing, robbery and hoverflies respectively, compared with the treated ones. For the plant extracts treatments, significantly fewer spiders (0.26± 0.03) and hoverfly larvae (0.09± 0.02) were recorded on the plots treated with *T. vogelii*. Fewer Robber flies were recorded in plots sprayed with *L. camara* (0.1± 0.04) and *T. vogelii* (0.2±0.03) compared with untreated plots (0.45± 0.05) and *T. diversifolia* (0.61± 0.15).

Table 5: Abundance of Beneficial Arthropods in Response to the Application of Treatments in Farmers' Plots in 2019 Cropping Season

Treatment	**Spider(adult)**	**Ladybird beetle (adult)**	**Hoverfly (larvae)**	**Ladybird beetle (larvae)**	**Lacewing (adult)**	**Robber fly (adult)**	**Hoverfly (adult)**
Control - u	0.38 ±0.04 a	0.39±0.07 a	0.16±0.02 b	0.11± 0.02a	0.45± 0.05a	0.45± 0.05a	0.38±0.04 a
T. diversifolia	0.27±0.06 ab	0.39±0.11 a	0.28± 0.03a	0.06± 0.02a	0.19± 0.05a	0.61± 0.15a	0.29± 0.05a
T. vogelii	0.26± 0.03b	0.21 ±0.04a	0.09± 0.02c	0.05±0.01 a	0.32± 0.05a	0.2±0.03 b	0.23±0.03 a
L. camara	0.3± 0.15ab	0.13± 0.1a	0.1± 0.06bc	0.03± 0.03a	0.15± 0.09a	0.1± 0.04b	0.2±0.1 a
ANOVA (F statistics)	2.7*	2.1 ns	6.1***	1.9ns	3.1ns	6**	3.7ns

The values presented are means ± SE. *= significant at P ≤ 0.05 ** = significant at P ≤ 0.01, * = significant at P ≤ 0.001 and ns = not significant. Means followed by the same letter in a column are not significantly different at P= 0.05 according to Fischer least significance difference (LSD)**

(ii) Treatment Effects on Pest Abundance, Grain Yield and Damage on Common Bean

On farmers' individual plots in the 2018 season (Fig. 11), significant variation in abundance of pests across treatments was recorded on aphids (ANOVA F = 12.4, df = 113, p = 0.0001), foliage beetles (ANOVA F = 12.6, df = 113, p = 0.0001) and pod suckers (ANOVA F = 2.7, df = 113, p = 0.03). The abundance of flower beetles (ANOVA F = 1, df = 113, p = 0.4) did not vary significantly across the treatments. The trend shows less abundance of aphids on *T. vogelii* treated plots and highest abundance on the untreated (Control –u). Again, there was no significant difference (P<0.0001) in the abundance of aphids among pesticidal plants and positive control. Highest damage was recorded on the untreated plots while minimum damage was observed in the plots treated with *T. vogelii*. Also, higher yield was recorded on *T. vogelii* while the lowest yield was on the untreated plots.

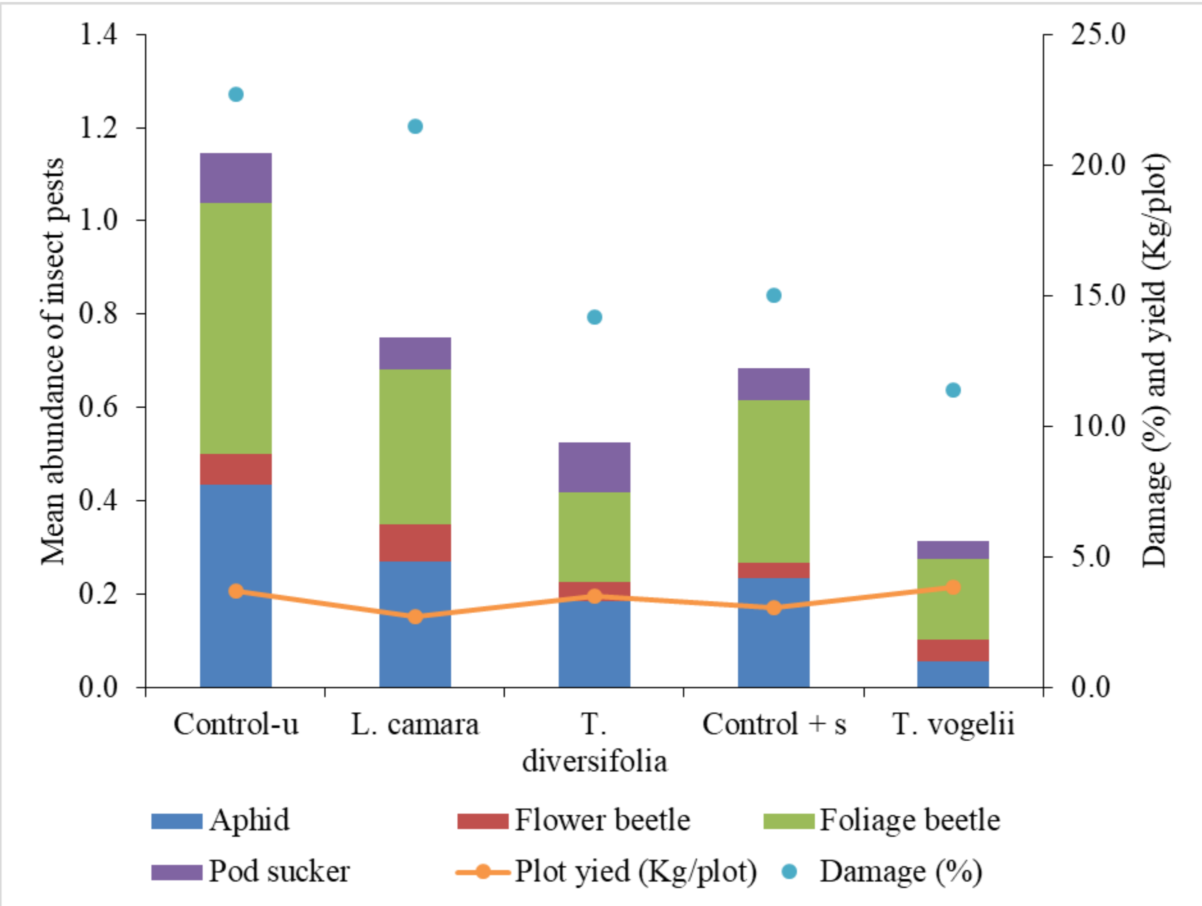


Figure 11: Treatment Effects on Pest Abundance, Grain Yield and Damage on Common Bean Farmers' Plots in 2018 Cropping Season

On demonstration plots (Fig. 12), the abundance of foliage beetles (ANOVA F = 5.8, df = 19, p = 0.01) varied across the treatments where significantly higher abundance was recorded on the untreated (Control –u) plots. Lowest damage was recorded on the *T. vogelii* treatment followed by synthetic pesticide and *T. diversifolia* while significantly (P<0.01) higher damage was recorded on the untreated (Control-u). Bean yield was significantly (P<0.0001) lower on the untreated plots compared with the rest of the treatments. Among treatments, however, there was no significant difference in grain yield in positive control and plant extracts.

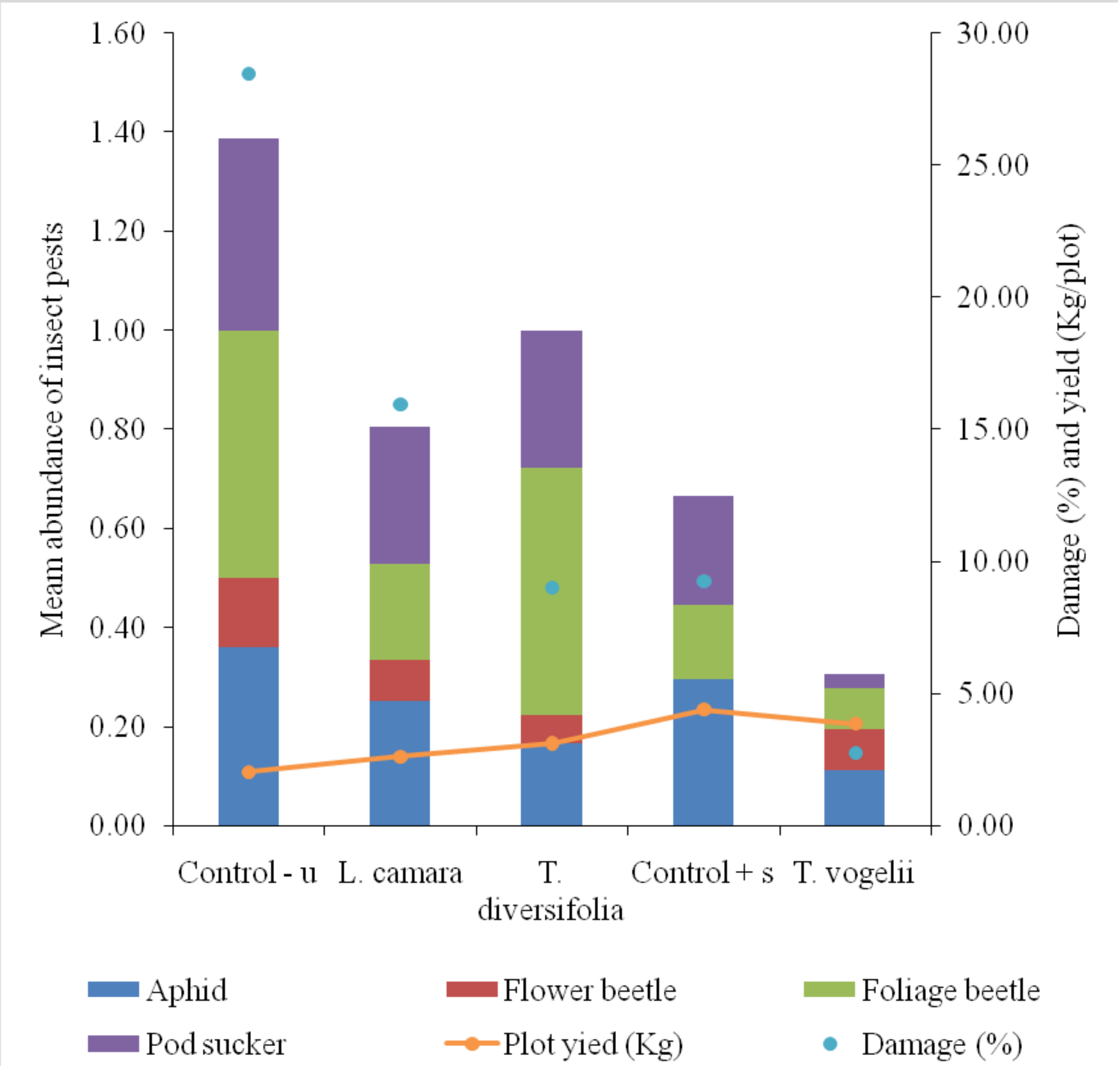


Figure 12: Abundance Insect Pests, Damage and Grain Yield of Common Beans per Plot in Demonstration Plots of 2018 Cropping Season

(iii) Influence of Treatments on Damage and Insect Pest Abundance on the 2019 Cropping Season

In the 2019 cropping season, significantly more damage (ANOVA $F = 11.3$, $df = 129$, $p = 0.0001$) was recorded in the untreated compared with the plant extract-treated plots (Fig. 13). The results in this season are a comparison between untreated and the plant extracts treatments only as this was farmers' preference. The abundance of aphids ($P<0.001$), foliage beetles ($P<0.0001$) and flower beetles ($P<0.001$) were significantly higher on the untreated plots compared with the untreated plots. In this season grain yield data was not recorded due to crop failure caused by severe drought.

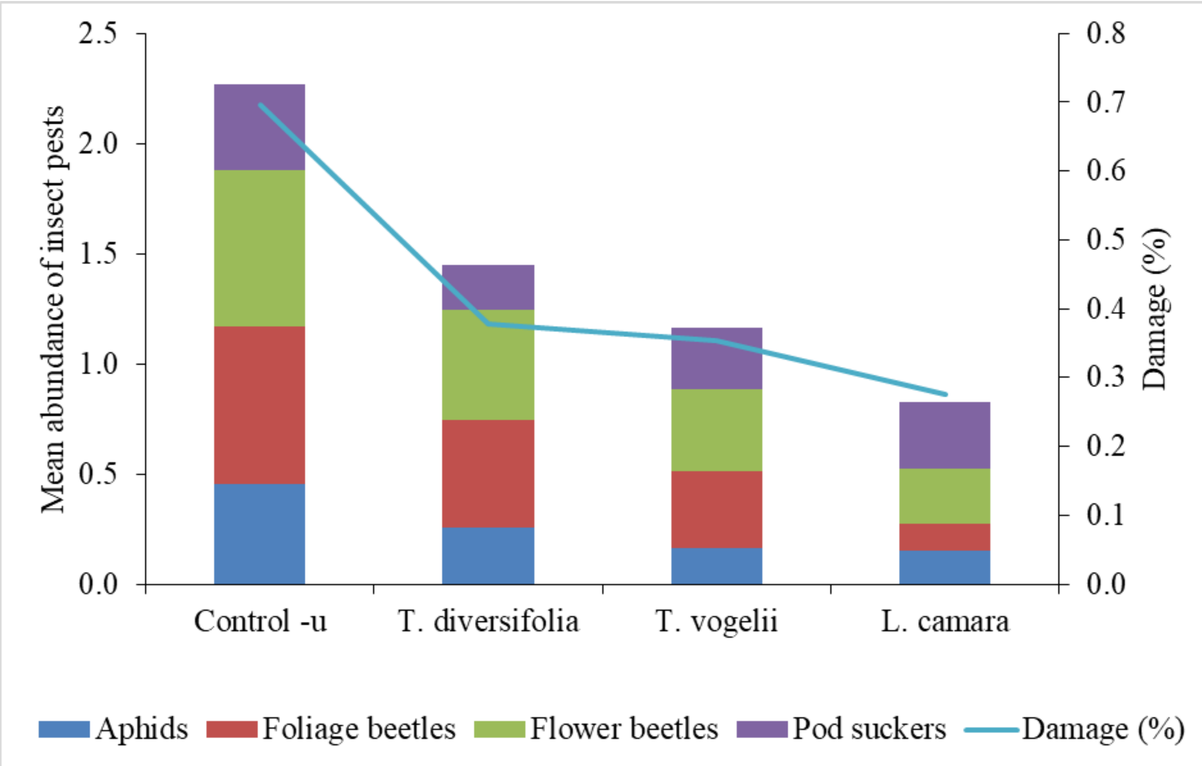


Figure 13: Impacts of Treatments in the Common Bean Insect Pest Abundance and Damage in the 2019 Cropping Season

4.1.3 To Characterize the Chemical Variation of *T. vogelii* by Seasons and Location on Production, Concentration and Chemotype of Pesticidal Compounds

(i) Status of Use of *T. vogelii* by Smallholder Farmers

Eight questions were used to assess the extent of *T. vogelii* use among farmers in locations where samples were collected in Tanzania. All interviewed farmers (n=22) were aware of *T. vogelii*, commonly known as “Utupa”. Farmers reported using *T. vogelii* mostly for field pest control (59%), fishing (45%), and storage pest control (36%). Additionally, uses such as human medication, soil fertility, as mole repellents, and for ectoparasites, control were also reported as shown in Fig. 14. A few farmers (5%) reported awareness of the plant from witnessing institutional researchers collecting the plant for use in research and also planting *T. vogelii* in research institutions such as the TARI - Uyole centre, Mbeya and Tanzania Coffee Research Institute (TACRI), Kilimanjaro. The specific research activity was not communicated. Results indicate that *T. vogelii* is most widely used for pest control among other uses, as shown in the survey results.

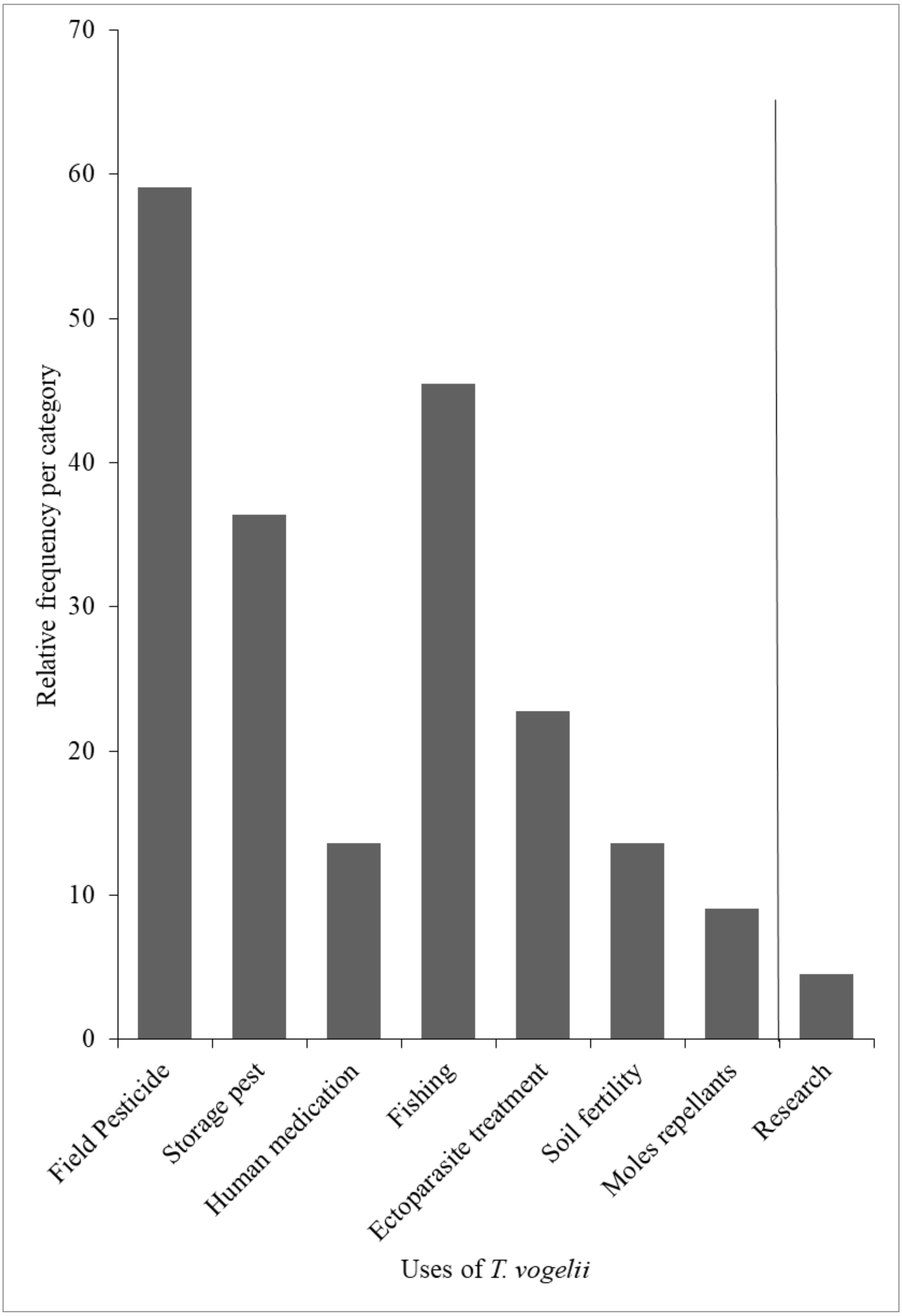


Figure 14: Ethnobotanical Uses of *T. vogelii* Among Local Smallholder Farmers from Six Tanzanian Regions

(ii) Phytochemical Analysis of *T. vogelii*

Analysis of methanolic extracts of the leaf samples identified 2 chemotypes. Figure. 15, shows the LC-MS chromatograms of chemotype 1 characterised by the presence of rotenoids with corresponding peaks between 19 and 20 min. These were determined from a comparison of their spectral data to in-house standards (Stevenson *et al.*, 2012). Rotenone was identified from UV (LC-PDA) λmax nm, 301; (MS) m/z, 395.4 [M + H]+, while tephrosin was identified from UV (LC-PDA) λmax nm, 272, 300, 314; (MS) m/z, 433.4 [M + Na]+ and deguelin from UV (LC-PDA) λmax nm, 270, 301, 319; (MS) m/z, 395.4 [M + H]+. Chemotype 2 (Fig. 16) with peaks between 18 and 21 min were determined to contain obovatin 3-O-methylether as the major component from an in-house standard and had UV (LC-PDA) λmax nm, 270, 295, 348; (MS) m/z, 337.4 [M + H]+. Other similar components having ions with m/z = 337 and 367 corresponding to flavones and flavanones are reported earlier, including Z-tephrostachin (Stevenson *et al.*, 2012).

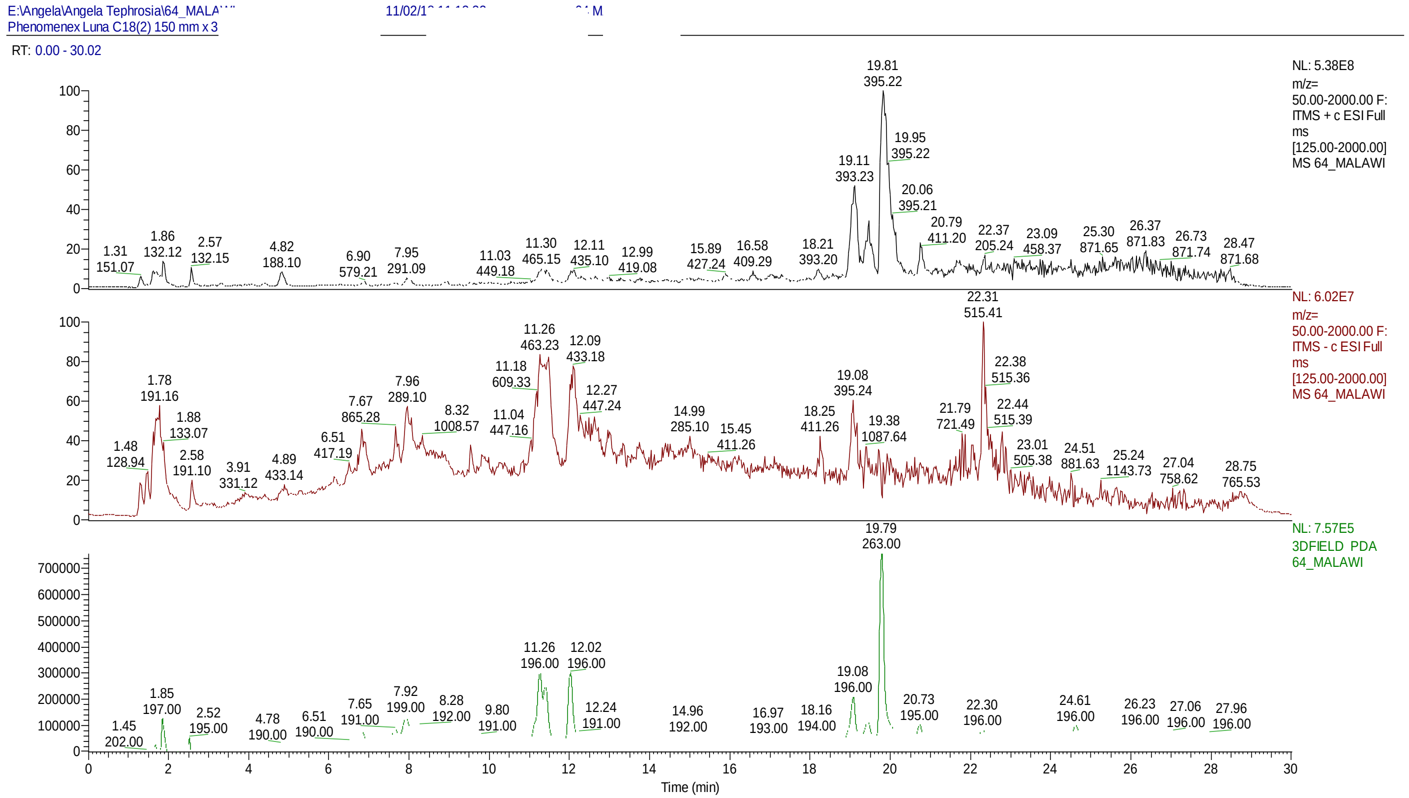


Figure 15: Chromatograms Showing Chemotype 1, Presence of Deguelin

A third chemotype was also identified (Fig. 17). Chemotype 3 was a chemical hybrid of chemotypes 1 and 2 showing the presence of both the rotenoids and the flavanones and flavones reported in 1 and 2 respectively in equivalent quantities. A further finding recorded plants as chemotype 1 but indicated trace quantities of flavones and flavanones from chemotype 2, suggesting that a variety of potential chemical variants may exist in natural and propagated materials. The analyses were undertaken on a single leaflet so were not a consequence of sample mixing of chemotypes 1 and 2. Furthermore, the individual leaves from the same plant were chemically very similar.

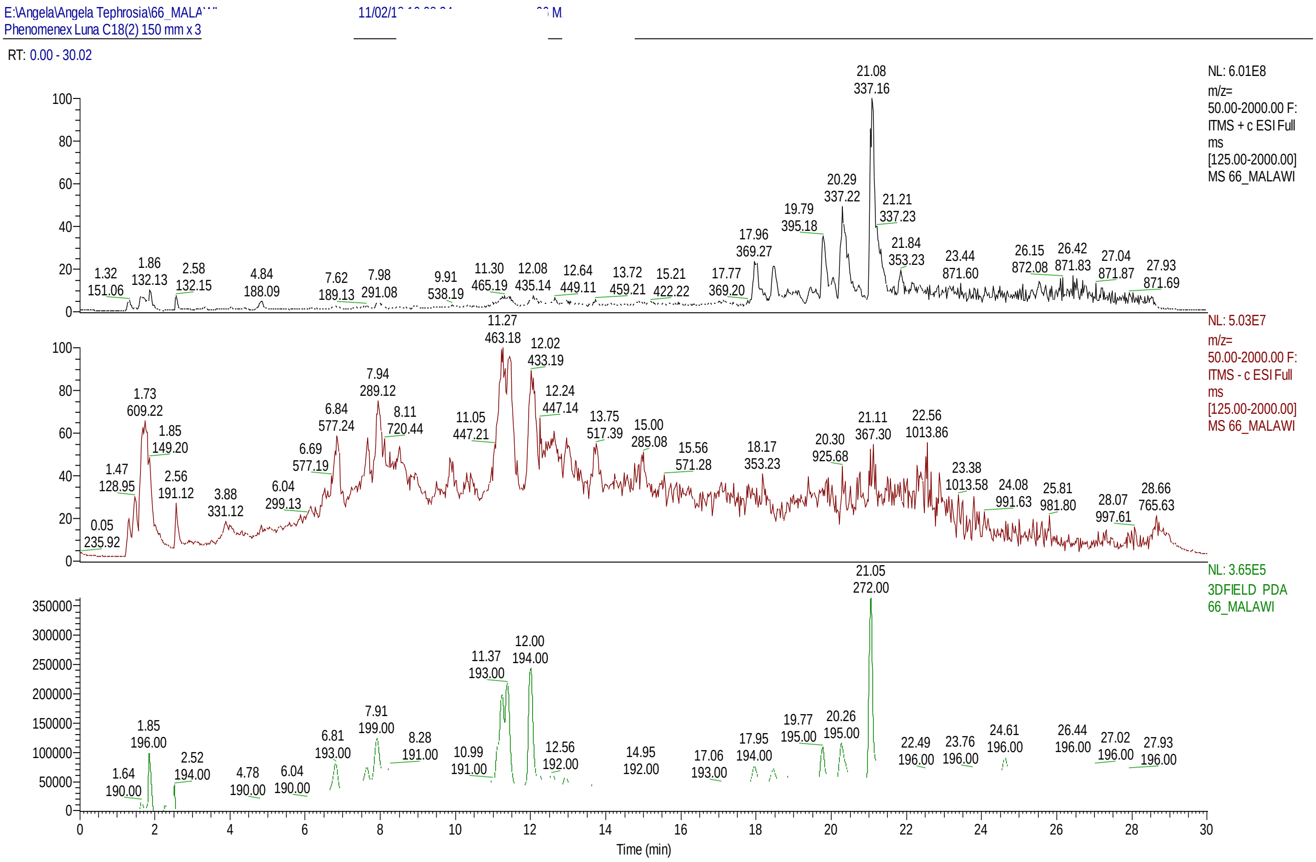


Figure 16: Chromatograms Showing Chemotype 2, Absence of Deguelin

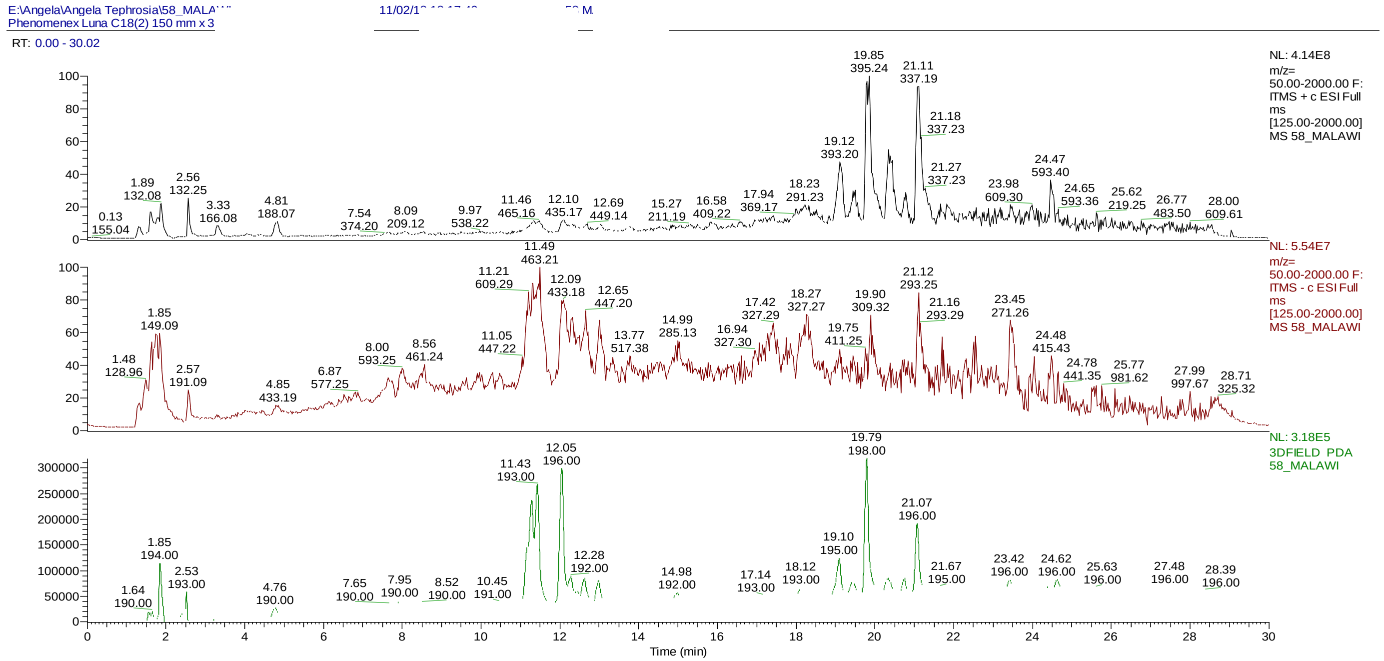


Figure 17: Chromatograms Showing Chemotype 3 Signifying the Presence of Chemotypes 1 and 2 Suggesting a Hybrid

(iii) Proportions of *T. vogelii* Chemotypes

Plant materials were collected from specific locations in three countries (Tanzania, Kenya and Malawi) where *T. vogelii* is used, and their geographical references are presented in (Appendix 1). Approximately, 7% of samples were identified as chemotype 3, while 20% were chemotype 2. Most samples (74%) were chemotype 1. Table 6 illustrates the proportions of chemotypes by countries.

(iv) Spatial Distribution of Plants Samples Chemotypes

In this study, deguelin, the most abundant pesticidal rotenoid in *T. vogelii*, was used as an indicator compound, and its concentration in the plant was assessed across the study zones. The chemical composition of *T. vogelii* was presented with reference to location across the three countries (Fig. 18). Samples collected from Malawi and Kenya contained chemotypes 1, 2 and 3 and were located in Lilongwe and in 12 Kenyan counties, respectively, while only chemotype 1 was recorded from 14 locations across five regions in Tanzania (Table 6).

The results from this study present the potential for understanding the diversity of pesticidal plant chemotypes across a whole region. Local efficacy testing of pesticidal activity in *T. vogelii* using a simple assay would potentially be conducted across various locations where the plants grow to ensure reliable efficacy results for local farmers using the plant.

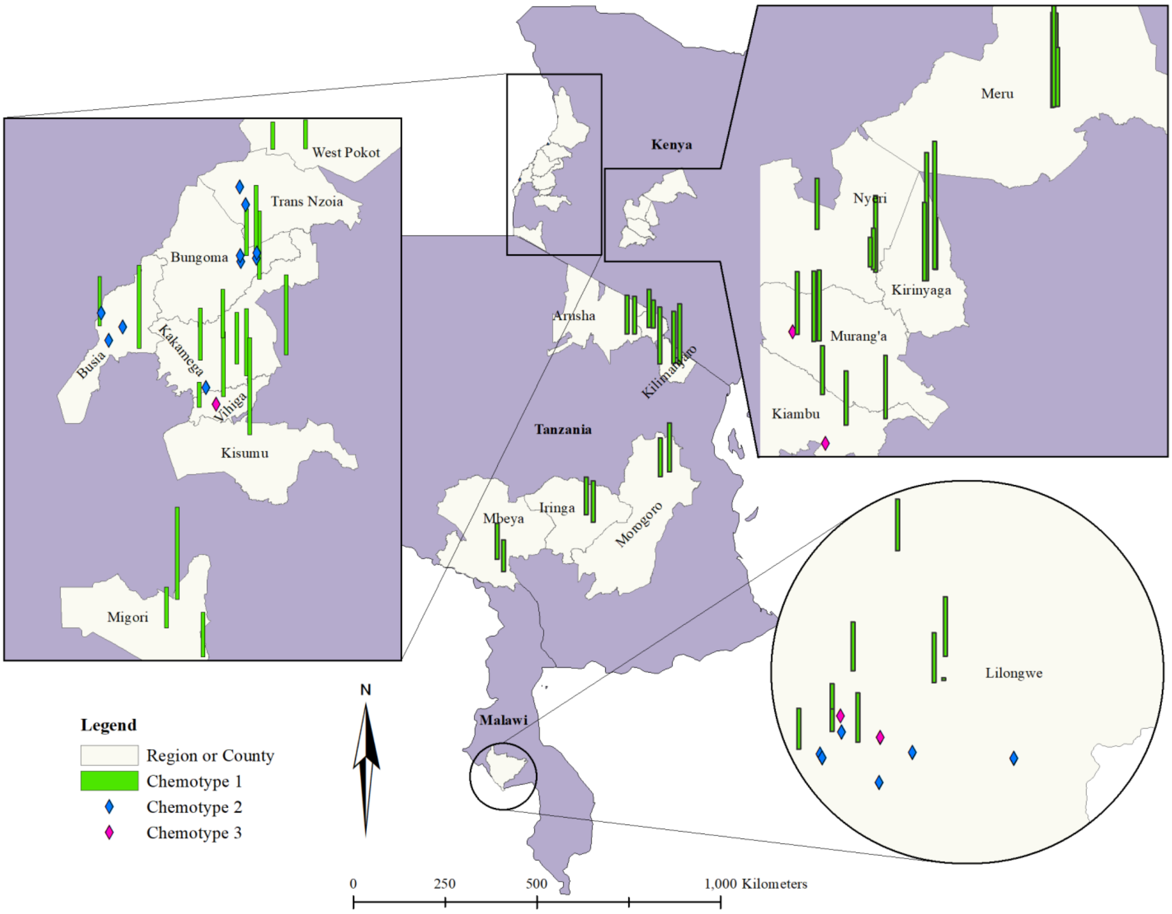


Figure 18: Spatial Variation of *T. vogelii* Chemotypes in Tanzania, Kenya, and Malawi Indicating the Presence of Chemotypes 1, 2 and 3. Green Bars Depict the Presence of Deguelin while Blue Marks Indicate the Presence of Chemotype 2. The Purple Marks Indicate the Presence of Chemotype 3

Table 6: Summary Distribution of Chemotype within the Study Area

Variables	No. of obser-vations	No. of missing values	No. of categories	Mode	Mode frequency	Categories	Frequency per category	Rel. frequency per category (%)	Proportion per category
Overall	91	0	3	Chemotype 1	67	Chemotype 1	67	74	1
						Chemotype 2	18	20	0
						Chemotype 3	6	7	0
Kenya	57	0	3	Chemotype 1	44	Chemotype 1	44	77	1
						Chemotype 2	10	18	0
						Chemotype 3	3	5	0
Malawi	20	0	3	Chemotype 1	9	Chemotype 1	9	45	0
						Chemotype 2	8	40	0
						Chemotype 3	3	15	0
Tanzania	14	0	1	Chemotype 1	14	Chemotype 1	14	100	1
						Chemotype 2	0	0	0
						Chemotype 3	0	0	0

(v) Spatial-temporal Variation of Chemotype 1 in *T. vogelii*

Linear regression analysis was performed to test the variation of deguelin content in *T. vogelii* based on altitude. The linear regression for Malawi ($r^2 = 0.178$, $F = 3.9$, $df = 18$, $p = 0.064$), Kenya ($r^2 = 0.03$, $F = 1.74$, $df = 56$, $p = 0.193$), dry season in Tanzania ($r^2 = 0.008$, $F = 0{,}096$, $df = 12$, $p = 0.762$), and wet season in Tanzania ($r^2 = 0.122$, $F = 1.665$, $df = 12$, $p = 0.221$) showed no significant relationship between changing altitude and the concentrations of deguelin. Further analysis of data from Tanzania revealed no significant correlation between rainfall recorded in the wet ($r^2 = 0.005$, $F = 0.016$, $df = 3$, $p = 0.9$), and dry seasons ($r^2 = 0.72$, $F = 7.725$, $df = 56$, $p = 0.069$). Analysis of variance on the samples collected over two seasons in Tanzania showed that there was no significant variation in the deguelin concentration with locations in the dry season (ANOVA $F = 0.272$, $df = 8$, $p = 0.916$). In the wet season, however, a significant variation (ANOVA $F = 7.092$, $df = 8$, $p = 0.008$) was observed (Table 7) where the highest and lowest levels of deguelin were observed in samples collected from Same and Mbeya districts, respectively.

Table 7: Spatial and Temporal Variation of Deguelin in *T. vogelii* from Locations in Tanzania

Location	Dry season Deguelin (ppm)	Wet season Deguelin (ppm)
Same	6841 ± 523 a	8756 ± 197 a
Iringa	5644 ± 1202 a	4879 ± 132 bc
Morogoro	5423 ± 1621 a	6229 ± 207 b
Kilimanjaro	5144 ± 682 a	6377 ± 791 b
Mbeya	5699 ± 314 a	3385 ± 196 c
Arusha	5339 ± 139 a	4803 ± 4 bc
One way ANOVA F statistics	0.27ns	7.09**

The values presented are means ± SE. **, = significant at P ≤ 0.01, ns = not significant. Means followed by the same letter in a column are not significantly different at P= 0.05 according to Fischer least significance difference (LSD)

(vi) Association between *T. vogelii* Flower Colour and Chemotypes

One simple morphological feature for identification of *T. vogelii* and potentially distinguishing chemotypes would be flower colour, with colours typically white or purple. In this study, plants that had flowers at the time of sample collection were recorded along with leaf samples for chemical analysis. A high percent of plants with white coloured flowers were recorded compared with those with purple flower (Fig. 19). A higher percent of occurrence of white flowers was associated with the presence of chemotype 1 (Fig. 20). Chemotype 3 was associated with only purple colour while a lower percentage of white colour was associated with chemotype 2 (Fig. 21). Regression analysis showed a strong correlation (r^2 = 0.43, F = 22.02, df = 29, p = 0.0001) between chemotype and flower colour where chemotype 1 was related to white and chemotype 2 to purple colour.

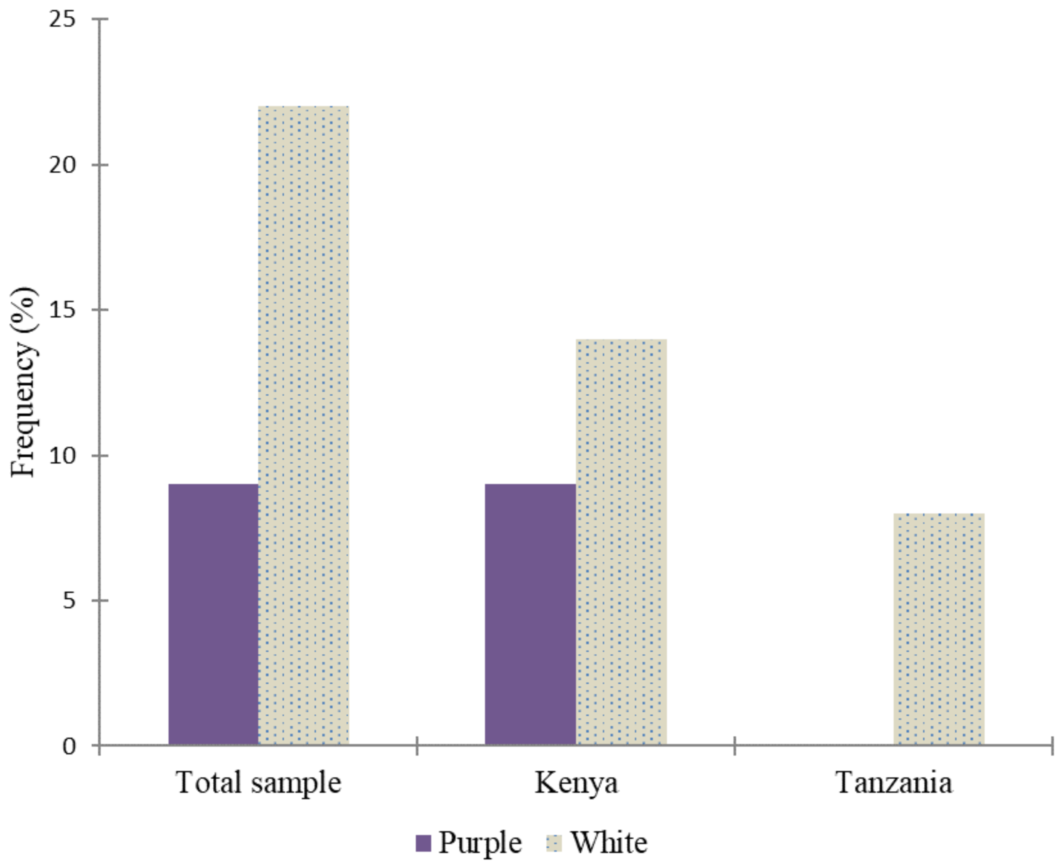


Figure 19: Status of Flower Colours by Countries

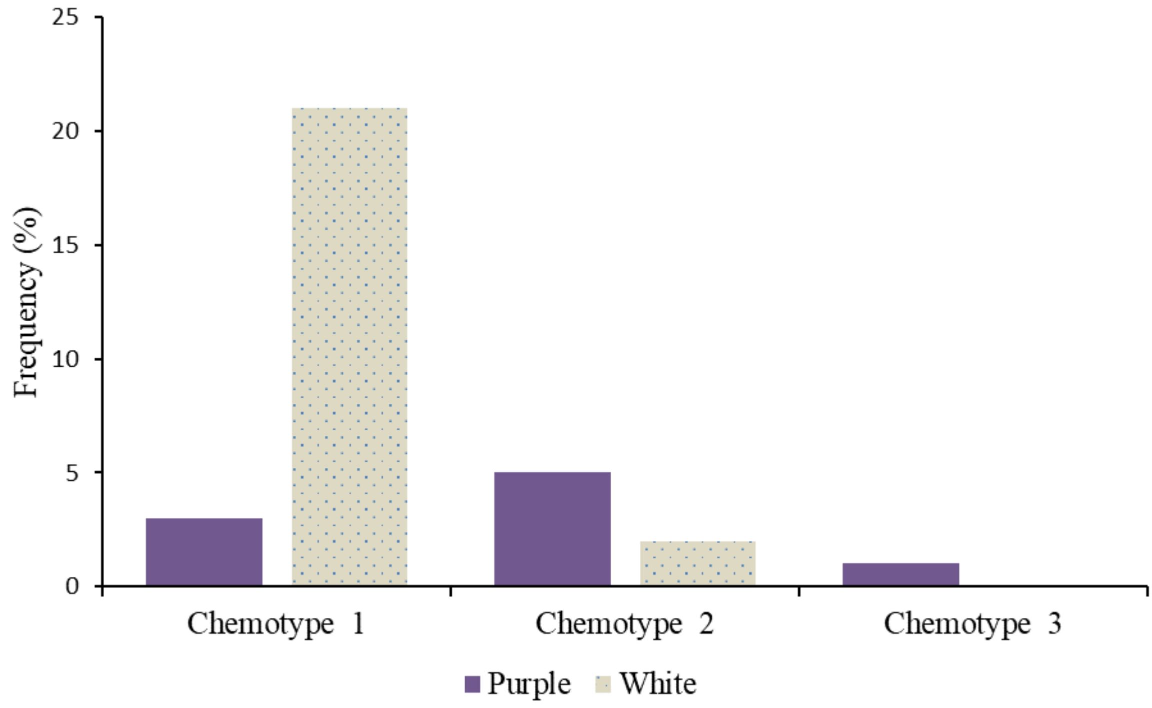


Figure 20: Association between Flower Colours and Chemotype of *T. vogelii* Materials

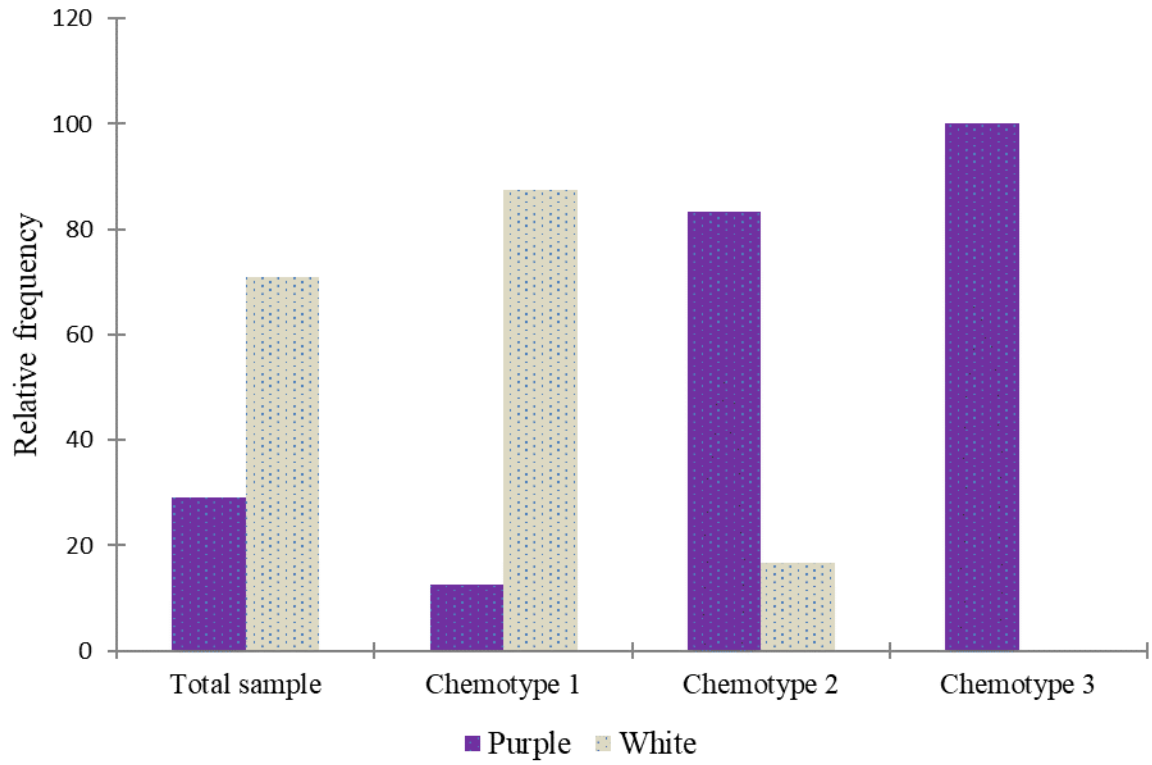


Figure 21: Relative Frequencies of Flower Colours with Respectively Associated Chemotype

(vii) Summary of Indicators for Chemotypes

Identification of the right chemotype by smallholder farmers require hands-on information enabling them to decide on suitable *T. vogelii* materials for pest control. From this study, proposed and tested indicators would be used. Table 8 below shows what farmers would need to consider while harvesting and using the materials.

Table 8: Tested and Proposed Options that Farmers Would Need to Consider when Selecting Effective *T. vogelii* Plant Material

Tested options	Result	Reliability for chemotype identification
Elevation		Not reliable
Season	No correlation	Not reliable: Although wet season enhances higher content of bioactive compounds in chemotype 1
Flower colour	Positive correlation	Somewhat reliable: Could be used to decide on the chemotype where white flowers are known to be related to chemotype 1. N.B., a few plants with chemotype 1 had purple flowers.
Proposed options		
Simple assays	Report from Belmain *et al.* (2012)	Reliable: Test assessment of plant (10% leaf powder in small test container with bruchids), could be a rapid, simple and affordable tool. Pesticidal properties of *T. vogelii* are fast-acting and chemotype could be determined in 48 h.

4.1.4 Evaluation of the Contribution of Foliar Application of Pesticidal Plants Extracts on Bean Plant Growth

(i) Growth and Grain Yield of Common Beans in Response to the Application of Treatments

Extracts were applied to the leaves through foliar spraying or directly to soil as a soil drench in order to compare the effects on bean plant growth and grain yield. Significant variation in the growth of common beans was observed according to treatments where *T. vogelii* extracts resulted in significantly higher plant height ($P<0.01$), number of leaves ($P< 0.5$) and branches ($P<0.001$), leaf area ($P<0.01$), stem width ($P<0.001$) and leaf greenness ($P<0.001$). However, water, water and soap, and synthetic pesticide treatments were significantly lower in terms of plant height number of leaves, a number of branches per plant, leaf area, stem width and leaf greenness (Appendix 2).

The grain yield was measured using the number of pods per plant and seed yield per plant (Table 9). Significantly, higher numbers of pods per plant ($P<0.001$) and seeds yield ($P<0.001$) were recorded in the *T. vogelii* treatment, followed by *T. diversifolia* and the foliar fertilizer for pods per plant and seed yield per plant. The control treatments (water, water and soap and synthetic pesticide) recorded significantly lower numbers for pods per plant) and seed yield. Both, the number of pods per plant and seeds per pod showed a significant variation with respect to the method of application with higher values recorded for the number of pods per plant ($P<0.05$) and seed yield per plant ($P<0.001$) when treated by foliar spray compared with when the treatments were applied to the soil for pod number and seed yield.

Table 9: Effects of Foliar Fertilizer, Synthetic and Plant Pesticide Treatments and Application Method on the Grain Yield of Common Beans

Treatment applied	**Number of pods/plant**	**Seed yield/plant (g)**
Foliar fertilizer	3.1±0.26 b	2.7±0.33 b
Synthetic pesticide	2.1±0.24 c	1.3±0.19 c
T. vogelii	4.1±0.23 a	3.8±0.23 a
T. diversifolia	3.1±0.31 b	3.3±0.23 b
Water	1.9±0.23 c	1.5±0.16 c
Water and soap	1.6±0.22 c	1.7±0.11 c
Method of application		
Foliar spray	2.9±0.21 a	2.7± 0.20 a
Soil drenching	2.4±0.16 b	2.1± 0.16 b
2-way ANOVA (F-statistics)		
Treatment	15.2***	29.0***
Treatment method	6.7*	14.8***
Treatment*Treatment method	2.0*	3.1*

The values presented are means ± SE. *, * = significant at $P \leq 0.05$, $P \leq 0.001$ respectively. Means followed by the same letter in a column are not significantly different at P= .05 according to Fischer least significance difference (LSD)**

(ii) Effect of Treatments and Application Method on Common Bean Metabolite Production

Analysis of chlorophyll content, flavonoids and anthocyanins indicated that the *T. vogelii* treatment resulted in significantly higher chlorophyll concentration ($P<0.001$), followed by the foliar fertilizer and *T. diversifolia* (Table 10). Lower chlorophyll content was observed in water, water and soap and the synthetic pesticide. Flavonoid content was highest in *T. diversifolia* treated plants, followed by the foliar fertilizer and *T. vogelii*, and these were significantly different from the water and water and soap treatments ($P<0.05$). No significant variation was observed in anthocyanin content across treatments or modes of application suggesting that the influence of treatments on plant metabolism was specific.

Table 10: Effect of Treatment on the Presence of Key Metabolite Groups in Common bean

Treatments	Chlorophylls (mg/l)	Flavonoids (Abs g DM−1)	Anthocyanins (Abs g DM−1)
Foliar fertilizer	19.3±1.84b	2.8±0.28ab	0.1±0.01a
Synthetic pesticide	13.7±0.74c	2.4±0.14bcd	0.1±0.00a
T. vogelii	24.6±1.29a	2.7±0.23abc	0.1±0.01a
T. diversifolia	18.9±0.89b	3.0±0.16a	0.1±0.01a
Water	12.7±0.53c	2.1±0.17d	0.1±0.03a
Water and soap	14.0±0.49c	2.2±0.15cd	0.1±0.02a
Method of application			
Soil drench	15.9±0.89b	2.5±0.12a	0.1±0.01a
Foliar spray	18.5±1.14a	2.6±0.13a	0.1±0.01a
2-way ANOVA (F-statistics)			
Treatment	27.8***	3.4*	0.6ns
Method of application	12.7**	0.5ns	0.4ns
Treatment*Method of application	3.0*	1.3ns	0.3ns

The values presented are means ± SE. *, **, * = significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively, ns=not significant. Means followed the same letter in a column are not significantly different at P= 0.05 according to Fischer least significance difference (LSD)**

Leaf samples were further analysed to identify the contribution of treatments on the amounts of specific metabolites, including primary metabolites (phenylalanine and tryptophan) and the secondary metabolite, rutin. An analysis of variance showed that these metabolites were higher when exposed to the foliar spray method of application in comparison with soil drenching for phenylalanine ($P<0.001$), tryptophan ($P<0.01$) and rutin ($P<0.001$)(Table 11). Overall, the foliar application was more effective in inducing changes in phenylalanine (Fig. 22), tryptophan (Fig. 23) and rutin (Fig. 25).

Table 11: Two-way Analysis of Variance on the Influence of Mode of Application on the Relative Abundance (mg/g dry weight) of Phenylalanine, Tryptophan and Rutin

Method of application	Phenylalanine	Tryptophan	Rutin
Foliar spray	43608.3±4557.06a	45478.3±5450.15a	15093.8±1675.05a
Soil drench	26209.9±2127.52b	26805.8±2566.88b	9342.5±895.06b
2-way ANOVA (F-statistics)	13.4***	10.3**	12.8***

The values presented are means ± SE. **, * = significant at P ≤ 0.01, P ≤ 0.001 respectively. Means followed by the same letter in a column are not significantly different at P= 0.05 according to Fischer least significance difference (LSD)**

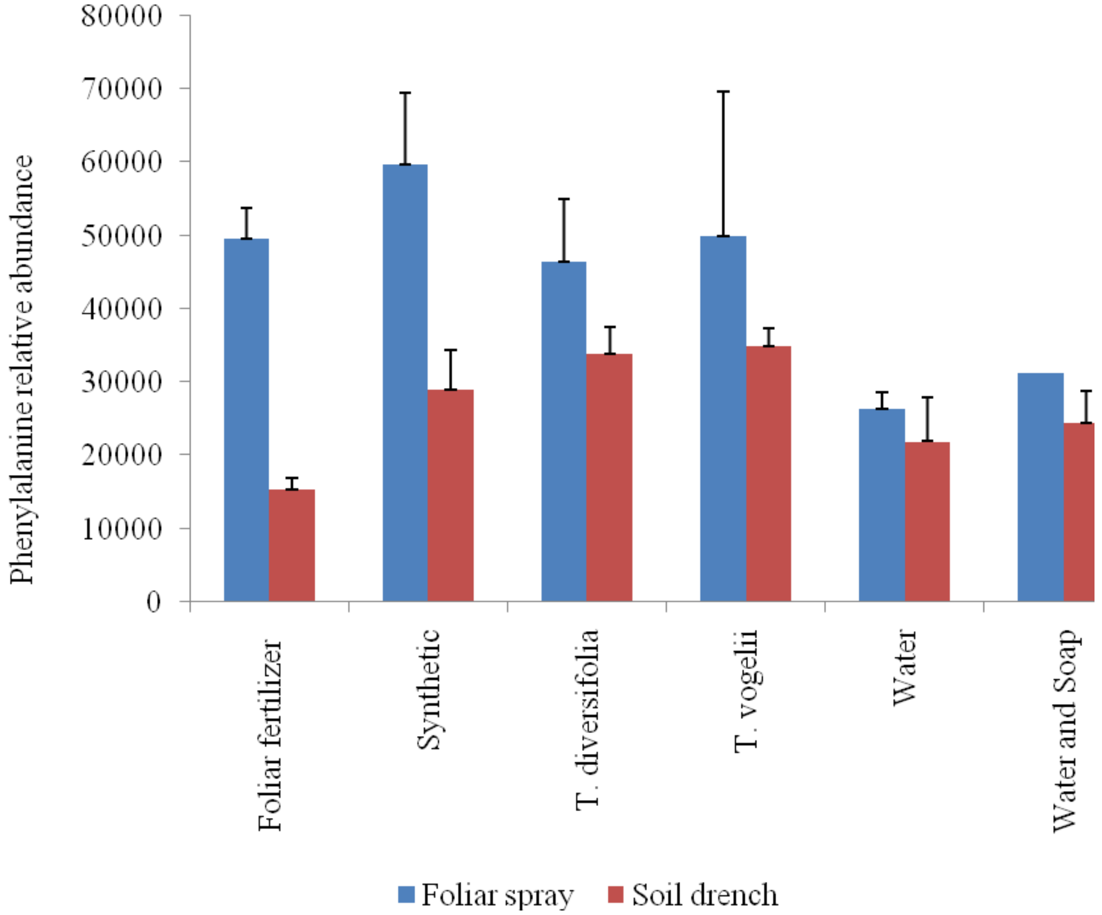


Figure 22: Relative Abundance (mg/g dry weight) of Phenylalanine in Common Bean Plants when Exposed to Different Experimental Treatments

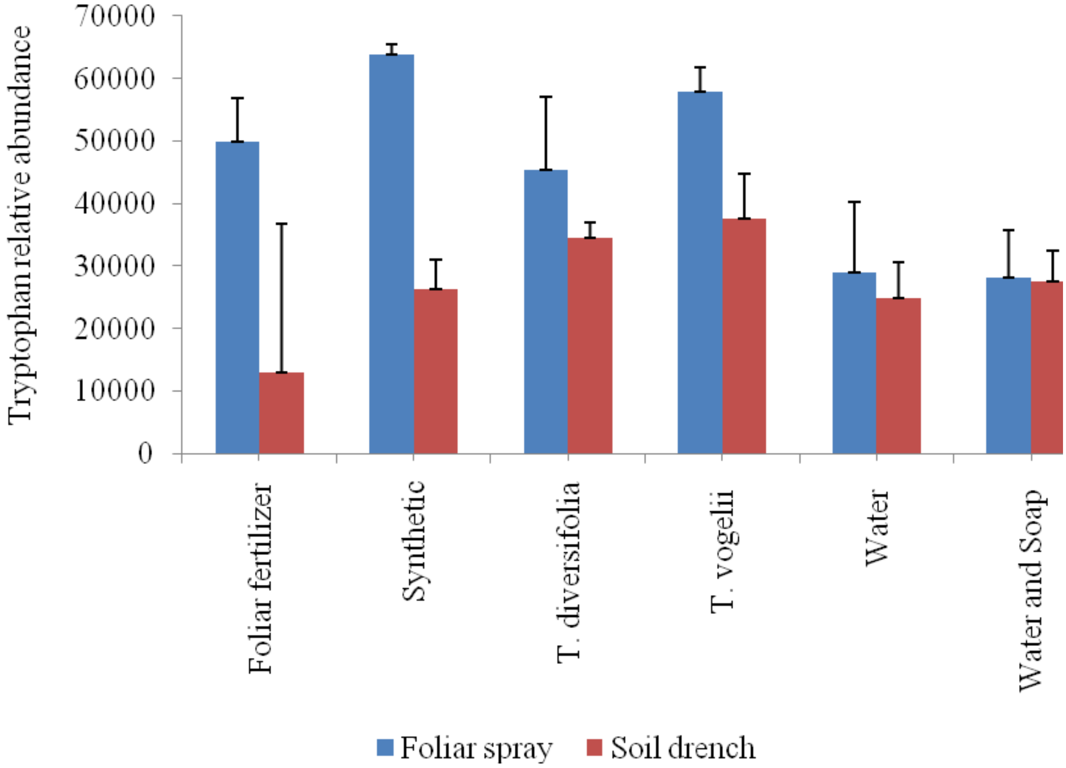


Figure 23: Relative Abundance (mg/g dry weight) of Tryptophan in Common Bean Plants when Exposed to Different Experimental Treatments

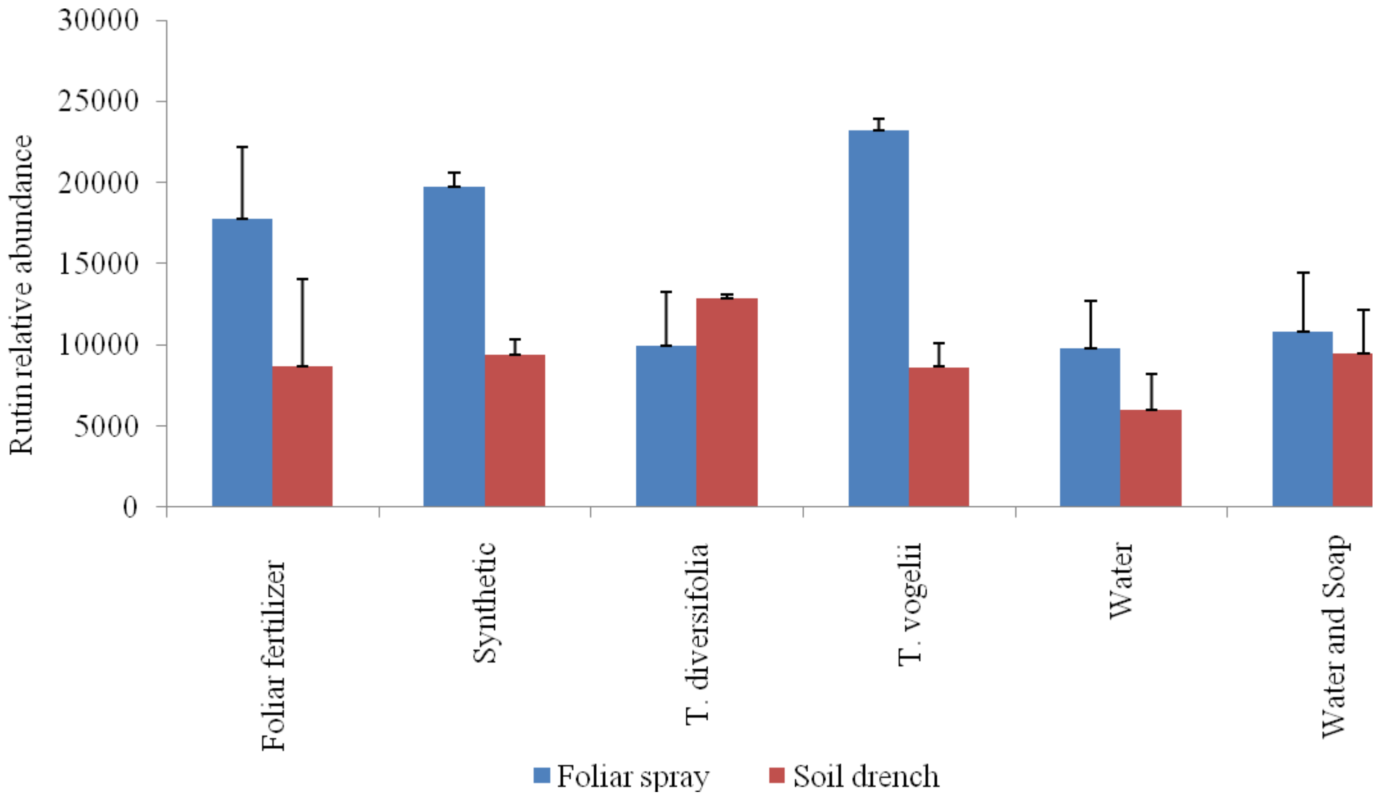
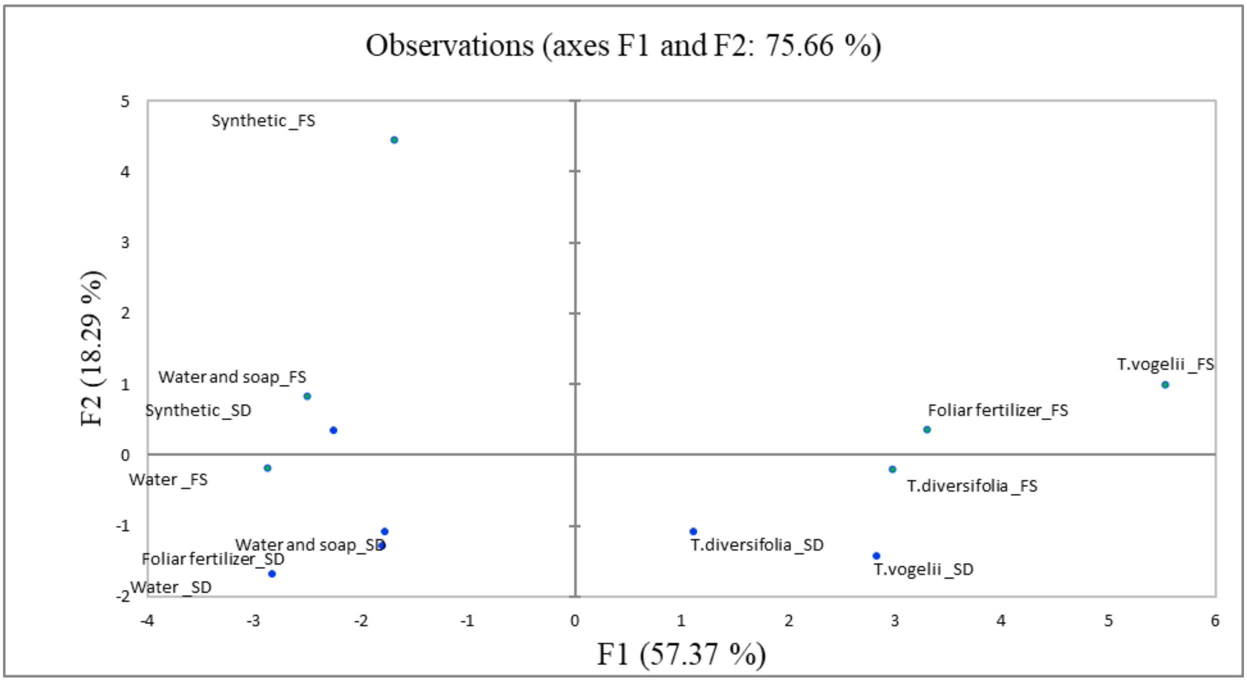


Figure 24: Relative Abundance (mg/g dry weight) of Rutin in Common Bean Plants when Exposed to Different Experimental Treatments

(i) Correlations Between Bean Plant Growth, Grain Yield Parameters and Common Bean Metabolites

Three principal components (PC1, PC2 and PC3) were retained to explain 87.2% variance of the dependent variables (Appendix 3-6). The criteria for selection were based on a cumulative variance of 70% and an eigenvalue greater than one. The first principal component accounted for a total variance of 57.37%, while the second and third components explained 18.3% and 8.7% of the total variance, respectively. Principal Component Analysis (PCA) observations of the treatments and their modes of application indicate the plant extracts applied to the bean plant, or the soil are grouped together, implying that their contribution to bean growth is related (Fig. 25a). Regardless of the plant extract species, application to the leaves had a negative relation with application to the soil. Treatments involving *T. vogelii* (Foliar spray) and water (Soil drench) showed the greatest and lowest influence, respectively. Furthermore, applying water had a low effect on the bean crop development regardless of the method of application.

Anthocyanin content correlated with the second principal component, which was different from the rest of the variables that all correlated with the first principal component (Fig. 25b). This difference is likely to be because anthocyanin values were minimal across all the treatments, with no significant difference observed in influencing bean development across the treatments. The first principal component's interpretation shows that yield parameters (number of pods per plant and seed yield per plant) and chlorophyll content explained more of the variation describing effects of the treatments. The number of branches showed a positive correlation with key metabolites, e.g. rutin (0.61), phenylalanine (0.58) and tryptophan (0.63).



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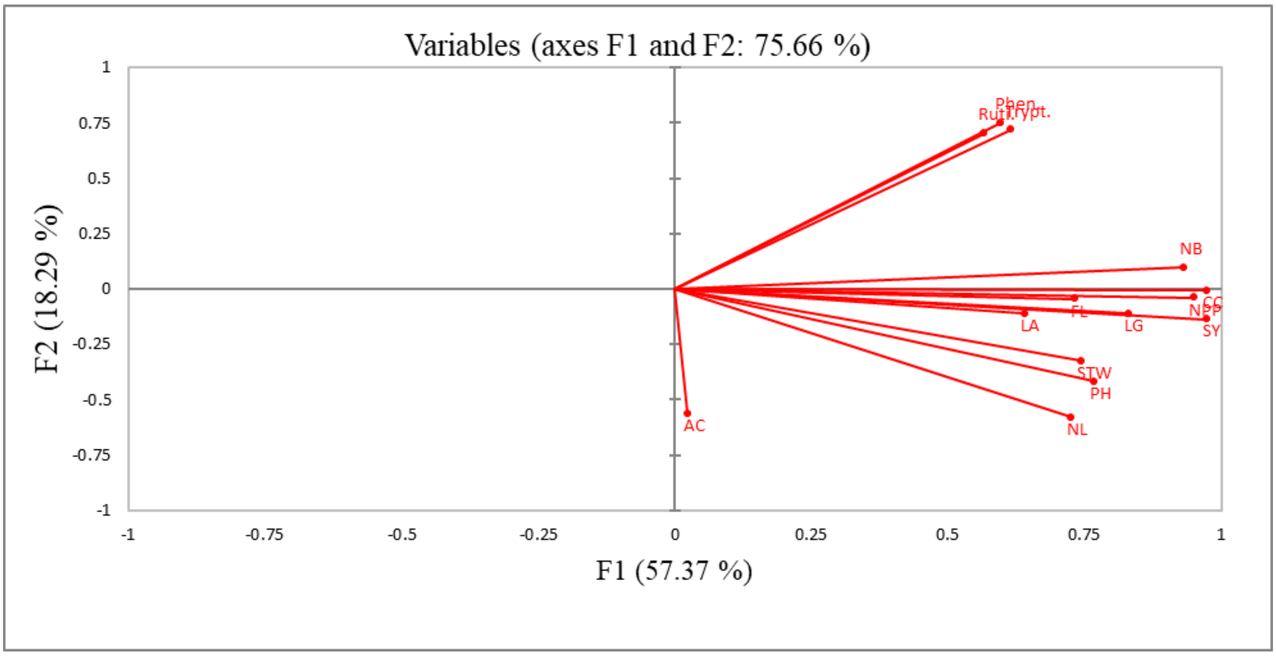

b

Figure 25: Two-dimensional principal component analysis (PCA) of treatments applied using foliar spray and soil drench methods

4.2 Discussion

This study has shown the potential of using plant extracts among smallholder farmers. Social study results have demonstrated the positive perception of farmers, towards the adoption of plant extracts use for pest management. Likewise, the regional wide phytochemical analysis has revealed spatio-temporal chemotypes variations in *T. vogelii* which support the existing research findings that inform its influence in the effectiveness of the plant and the adoption of pesticidal plant extracts use. Finally, the study has reported scientific evidence on the possibility of plant extracts contributing to bean crop growth promotion.

Results from the questionnaire survey confirm that plant extracts have been used for decades, although with inadequate validation and reproducible methods. Local preparation and use of pesticidal plants is a common practice as also reported by Dougoud *et al.* (2019), and it is known to have been practised in the past generations as published by Pavela (2016). From the questionnaire survey, results showed that plant extract use was practised in rural areas and mainly by the older generation (older than 50 years of age). Conversely, younger participants in the survey preferred using synthetic pesticides, confirming that old age preferred plant extracts more than the younger ones. Less preference in using older technologies by the younger generation was also identified by Abatania *et al.* (2009) who reported younger generation's preferences for the use of newer technologies, in this case, the use of synthetic chemicals.

Using synthetic pesticides is realized since the 1940s and has been practised more compared with the use of natural products. Use of synthetic pesticides in the study area was more practised by younger generations and was related to the influence of extension service, which only conveyed pest management using industrial chemicals. Farmers reported using synthetic chemicals more as the most popular intervention even when no expert directed. However, expertise on the alternative pest management options was limited as activities related to this research were the only reported interventions on natural pest regulation. In addition, the small percentage of farmers who received the extension services reported that about synthetic the received advise was mainly on synthetic pesticides and fertilizers and not any services on natural products. Abatania *et al.* (2009) reported extension services to influence the adoption of the technologies because they directly communicated agronomic practices to farmers. Hence, fewer extension services related to pest control using plant extracts present a challenge in the adoption of the technology.

From the FGD, farmers highlighted challenges, benefits and the ways forwards towards using the plant extracts. Challenges of using plant extracts in this study were regarded as the factors that prevented the adoption of the technology. Farmers reported that tools for harvesting, processing and application of plant extracts were inadequate. These elaborated findings resonate well with the cost-benefit analysis conducted by Baidoo and Mochiah (2016), who reported labour as a cost hindering the use of plant extracts. Other studies, such as a report from Ngbede *et al.* (2014) showed that use of pesticidal plants in the control of cabbage reported lower costs when using pesticidal plants than the use of synthetic pesticides. This finding reveals that, in practice, challenges of using plant extracts are existent, although not calculated on a monetary basis. A finding presented by Dougoud *et al.* (2019) further acknowledges the fact that plant extracts were a better alternative to synthetic pesticides although genuine challenges such as processing methods, varying active ingredients and less effectiveness existed. This study was able to identify farmers' opinions on the challenges of using plant extracts, which could be used as a reference for addressing pesticidal plants use problems in the future.

Benefits of using plant extracts shared out in this study included less harm to human health, accessibility of plant materials, the safety to the ecosystem and pest management potentials. These benefits are also reported widely in literature (Isman & Grieneisen, 2014; Mkenda *et al.*, 2015; Mkindi *et al.*, 2017; Isman, 2017; Stevenson *et al.*, 2017; Tembo *et al.*, 2018). In particular, the contribution of plant extracts use in safeguarding the ecosystem is reported by Amoabeng *et al.* (2019). Other benefits are also reported by Mabo and Cosentino (2018) from a review of potentials of *T. diversifolia* as a medicinal plant, showing that it was used to treat wounds, skeletomuscular disorders, abscesses, dermatological conditions, stomach pain, oral administration for diabetes, malaria, fever, hepatitis and infectious diseases. These findings coincide with farmers' information from the study area that plant extracts were also used as human disease cure and that by being natural, plants were regarded as harmless. The harmlessness of the plant extracts was explained by farmers who reported that it was not necessary to take antidotes after spraying plant extracts to treat chest pain, an effect linked with effects acquired from spraying synthetically made chemicals whereby drinking milk is often used as an antidote (Lekei *et al.*, 2014). Accessibility of plant materials was reported in this study as a critical benefit because plant materials were obtained in the farmer's premises in appreciable quantities. Plants such as *T. diversifolia* and *L. camara* spread quickly, thriving in various ecosystem types (Gooden *et al.*, 2009; Oke *et al.*, 2011) and are termed as invasive. Hence, the use of such plant material as pesticides would assist in suppressing their spread. On the other hand, a pesticidal plant like *T. vogelii* is a native of Africa that farmers can propagate, such as the practice in the study area. Another benefit of using plant extracts was a contribution to the growth of the crops. Farmers reported beans been greener when treated with plant extracts. This finding can be related to information from Munthali *et al.* (2014) *and* Jama *et al.* (2000) who reported *T. vogelii* having nutrient values to the food crops.

Use of plant extracts by smallholder farmers is practically demonstrated in this study where farmers established experimental trials to evaluate the efficacy of plant extracts for pest control. Overall results show that plant extracts can significantly control pests compared with the untreated ones. Evaluation results concur with previous studies which confirmed pesticidal plants as useful for pest management (Mkenda *et al.*, 2015). Also, results from farmers' trials confirmed that plant extracts posed less impacts to beneficial arthropods compared with the synthetic pesticides as also reported by Tembo *et al.* (2018). Among the tested pesticidal plant species, *T. vogelii* was found to be the most preferred where many farmers opted to use it for their experiments. Preference of *T. vogelii* could have emanated from the previous study in the same area where farmers evaluated *T. vogelii* as the most effective plant extracts (Mkindi *et al.*, 2017).

Using pesticidal plants is accompanied by inherent factors known to influence their efficacy and adoption by users. This study evaluated the phytochemical variation of *T. vogelii* based on the fact that farmers used the plant as the most preferred pesticidal plant species for field and storage pest control (Belmain *et al.*, 2012; Mkindi *et al.*, 2017). Use of *T. vogelii* is not only limited to pests in farming activities but also as ectoparasites control in domestic animals (Christopher *et al.,* 2009; Dougoud *et al.,* 2019; Kalume *et al.,* 2012). The broader use of *T. vogelii* for smallholder farmers could be associated with previous projects that promoted integrated pest management using *T. vogelii* and research on soil improvement (Mihale *et al.,* 2009; Snapp *et al.,* 2002).

Phytochemical variation analysis in this study focused on the presence of deguelin, a rotenoid which is reported to be an effective and abundant compound in *T. vogelii*. Stevenson *et al.* (2012) reported a presence of two chemotypes in *T. vogelii* from a collection of samples from Malawi. The findings correlated with a comprehensive regional assessment chemotype in this study which found out the presence of the two chemotypes in addition to one new type. The occurrence of chemotype 2 might have an implication on the application and uptake of this species for pest control, as reported by Belmain *et al.* (2012). Chemotype 3 is previously unreported in this species which attacks further investigations on its potentials and occurrence. There would, therefore, be value in analyzing for further chemotypes and determining if the hybrids produce lower quantities of both compound groups. Studies have shown that the production of new flavonoids such as those found in *T. vogelii* could be influenced by environmental factors whereby compounds changed after exposure to conditions such as carbohydrates and light (Lambert *et al.,* 1993). However, chemical variation in these varieties is most likely genetic since different chemotypes were first reported from the same location and in the same soil in adjacent fields (Stevenson *et al.*, 2012).

Further, analysis of chemotypes revealed that chemotype 1 was in higher proportions compared with other chemotypes. A higher percentage of chemotype 1 was also reported by Belmain *et al.* (2012) in Malawi from the analysis of 12 samples. The abundance in plant materials with chemotype 1 coincided with efficacy studies of *T. vogelii* on medicinal (Negi, 2012; Marango *et al.*, 2017; José Eduardo dos Santos *et al.*, 2019; Lokhande *et al.*, 2019) and insecticidal properties of rotenoids (Belmain *et al.*, 2012; Mkenda *et al*., 2015; Mkindi *et al.*, 2017; Tembo *et al.*, 2018) which revealed that rotenoids were the compounds most frequently found in *T. vogelii* sampled and are responsible for the plants' activity. From this analysis, variation with location and season did not influence the chemistry of deguelin, although wet season contained higher concentrations. The results concur with findings reported by Belmain *et al.* (2012), although these earlier data were of just a few samples. Seasonal variation of deguelin was also reported by Irvine and Freyre (2010) and Belmain *et al.* (2012) where higher concentrations occurred in the wet season compared with the dry season.

Because phytochemical analysis revealed chemical variation, smallholder farmers who may not have access to such analyses require easy and effective ways of ascertaining plants with the right chemotype. In this study, flower colours correlated with the chemotype where white colours were associated with chemotype 1, a contrast to earlier findings (Stevenson *et al.*, 2012) that found no correlation. Flower colours could be used as a primary tool for identification of chemotypes. However, the fact that some purple flowers (although in smaller per cent) were also associated with chemotype 1, the decision to use a plant for pest control purposes should be guided with simple assays for evidence of effective chemotypes. Smallholder farmers could adopt simpler tests of plant materials against storage pests such as cowpea weevils (*Callosobruchus maculatus*) as already done by Belmain *et al.* (2012), although this was under laboratory settings.

This study also evaluated the contribution of plant extracts to the growth and yield of common beans. Plant species tested, namely *T. diversifolia* and *T. vogelii* were observed to enhance the production of primary and secondary metabolites relative to the negative control. From the study, primary metabolites including tryptophan and phenylalanine, and secondary metabolites, including flavonoid, namely rutin, were enhanced in beans in response to the application of treatments. These findings were similar to those observed with Neem (*Azadirachta indica*), where similar metabolic changes were reported by Paul and Sharma (2002). Similarly, Neem extracts applied to tomatoes have been observed to increase the abundance of several flavonoids through the jasmonate pathway (Pretali *et al.,* 2016). Primary and secondary metabolites in plants can contribute to the development and growth of crop plants (Pretali *et al.*, 2016) as well as contribute to plant defence mechanisms (Bohinc *et al.,* 2012). Flavonoids are known to help a plant relate with other organisms and the environment thereby responding to biotic and abiotic stress (Mierziak *et al.*, 2014; Khalid *et al.*, 2019). Their contribution to growth is explained by their effect on growth, nodulation and yield of beans (Hussain *et al.*, 2011). Hence, applications that increase such metabolites in common beans could be beneficial to provide sustainable production techniques for bean resistance to pests, growth and yield as reported for ginger (*Zingiber officinale*) (Grzywacz *et al.,* 2014). From the study, applying *T. vogelii* and *T. diversifolia* was found to contribute to the increased presence of primary and secondary metabolites in bean plants. As expected, commercial foliar fertilizer had a significant effect on metabolite production. Additionally, plant extracts were found to influence chlorophyll content and the plants' greenness. The effect of *T. diversifolia* on chlorophyll content is also supported by previous research by Oke *et al.* (2011).

Additionally, this study also evaluated the contribution of methods of application of plant extracts and the control treatments on common bean growth and metabolites production. Results showed that foliar spray was more effective compared with the use of the extracts on the soil. Based on such findings, the data suggested that the plant extracts contribute to plant nutrition as a foliar fertilizer, which may be particularly useful in smallholder farming systems where soils are often degraded. Furthermore, these data suggest that previous reports on the use of these pesticidal plants in crop protection (Mkindi *et al.,* 2017; Tembo *et al.,* 2018; Kayange *et al.*, 2019) have maintained crop yield not only by fighting pests but by functioning as a foliar fertilizer. Contribution to growth and yield is likely related to the addition of phosphorus and nitrogen (Mafongoya *et al.,* 2003a) where *T. diversifolia* (Endris, 2019;

Pavela *et al.*, 2018) and *T. vogelii* (Snapp *et al.*, 2002; Rutunga *et al.*, 2008; Munthali *et al.*, 2014) are known to produce nitrogen-rich green biomass respectively.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study has identified factors that influence the adoption of plant extracts use by small-holder farmers. It has emphasized that knowledge of challenges, benefits and future plans towards using plant extracts is well understood when there is a participatory discussion and common understanding, which is context-specific as opposed to general assumptions and hypotheses. In this study, ways forwards to the enhanced use of plant extracts such as tools, education and plant domestication are identified. Likewise, the study has shown the efficacy of plant extracts compared to untreated plots and that *T. diversifolia* and *T. vogelii* are more efficient pesticidal plants relative to *L. camara*. In addition, farmers' participation in the whole field trials experiments processes in collaboration with researchers has been demonstrated using FRN approach, and have proved to facilitate extensive commitment in a short period of time.

The study has demonstrated the existence of chemical variations in *T. vogelii*, presenting chemotypes 1, 2 and 3, across agro-ecologies in three East African countries and thus reporting a variation in chemistry which influence the bioactivity of the plant. In this study, also, foliar sprays of the pesticidal plants, namely *T. vogelii* and *T. diversifolia*, enhanced common bean growth, production of metabolites and grain yield. Hence, using plant extracts for crop production can help farmers move towards more sustainable agroecological approaches to crop production, tackling both pest management and growth promotion using plant extracts.

5.2 Recommendations

(i) Participatory and collaborative experimentation and evaluation of research outcomes is an ideal approach that enhances broad uptake of viable technologies. Particularly FRN approach used in this study could be incorporated in scientific studies as a core of the research to foster active uptake of identified technologies after the accomplishment of research works. This is because, an FRN approach enhances farmer-researcher collaboration, farmer to farmer learning and sharing of information more extensively.

(ii) This research has elaborated challenges, benefits and future plans towards effective use of plant extracts in crop production. Hence, efforts to disseminate the obtained results are required in order to make the information reach out to more farmers in wider coverage.

(iii) The research has shown phytochemical variation in *T. vogelii,* which is known to influence the effectiveness of the plant in pest control. Importantly, the methodology for identifying such variation in this study was expensive and of high technology, for smallholder farmers to perform. Hence, to mitigate the variations under local conditions, simple and locally tailored assays, where farmers could test plant materials on storage pests would provide a rapid assessment tool to identify effective plant materials.

(iv) During this study, propagation of *T. vogelii* was observed among smallholder farmers. It is hence recommended that emphasis on using seeds from effective plant materials is made by researchers in order to make sure that propagation of elite materials is achieved.

(v) The results from this study showed a strong association between effective *T. vogelii* (Chemotype 1) with white colour, signifying that flower colour can be used as an indicator of the presence of effective plant materials. However, few effective *T. vogelii* were observed to have a purple colour. Hence, it is recommended that further research on indicators for the effective plant materials be conducted to help further with morphological identification of effective materials that suit smallholder farmers.

(vi) This study has revealed a third chemotype, chemotype 3, in addition to the previously reported chemotypes 1 and 2. Investigation of activity and other potentials of chemotype 3 is required along with studying factors that influence the presence of this chemotype in *T. vogelii* plant.

(vii) The study has shown the potentials for *T. vogelii* and *T. diversifolia* in growth promotion of common beans. Hence, the propagation of these plant species is important to ensure their availability and abundance. However, *T. diversifolia* is reported to be an invasive species, although the literature has shown no impacts associated with its invasiveness. Therefore, care is needed when considering the propagation of *T. diversifolia* or maintaining it so that it is kept under control because of its invasive nature.

(viii) This study was conducted on common beans. However, based on its observed potential, research works on other crops such as coffee, maize and banana, would enhance more understanding of potentials for pesticidal plants in wider crop production scopes.

(ix) The fact that plant extracts are effective under field conditions, and that smallholder farmers have collaborated in assessments of their efficacy, strategies for the establishment of commercialization is important in order to foster income generation from such resources.

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APPENDICES

Appendix 1: Locations for Samples of *T. vogelii* Collected in Tanzania, Kenya and Malawi

Farmer/Sample ID	Country	Latitude	Longitude	Altitude
01-K	Kenya	0.097752	37.81782	1168
02-K	Kenya	0.325643	34.53851	1340
03-K	Kenya	0.979937	35.15177	1892
04-K	Kenya	0.090201	37.80688	1171
05-K	Kenya	-0.526584	37.14551	1663
06-K	Kenya	1.011569	34.81895	1944
07-K	Kenya	0.428555	34.83557	154
08-K	Kenya	-1.10017	34.50181	1367
09-K	Kenya	0.498218	34.12219	1141
10-K	Kenya	-0.559879	37.3352	1314
11-K	Kenya	-0.750621	36.83641	2208
12-K	Kenya	0.873637	34.87782	1747
13-K	Kenya	-0.95086	34.32468	1274
14-K	Kenya	-0.759193	36.85265	2165
15-K	Kenya	-0.367204	36.92607	1865
16-K	Kenya	0.049257	34.68665	1561
17-K	Kenya	-0.558601	37.32853	1300

Farmer/Sample ID	Country	Latitude	Longitude	Altitude
18-K	Kenya	0.757884	34.86166	1662
19-K	Kenya	-1.167437	36.95979	1510
20-K	Kenya	0.432866	34.22514	1177
21-K	Kenya	0.437587	34.11315	1184
22-K	Kenya	0.743374	34.78629	1619
23-K	Kenya	0.115114	34.63049	1474
24-K	Kenya	0.36835	34.15812	1156
25-K	Kenya	0.112597	34.62899	1478
26-K	Kenya	0.110225	34.81027	1639
27-K	Kenya	0.211953	34.82631	1530
28-K	Kenya	-0.517841	37.37005	1413
29-K	Kenya	-1.074544	37.1823	1452
30-K	Kenya	1.098055	34.78232	2137
31-K	Kenya	1.014987	34.81005	1997
32-K	Kenya	1.176398	34.93681	1869
33-K	Kenya	-1.098766	37.03414	1504
34-K	Kenya	-1.099615	34.43201	1342
35-K	Kenya	0.257286	34.76586	1475
36-K	Kenya	-0.517195	37.13713	1684

Farmer/Sample ID	Country	Latitude	Longitude	Altitude
37-K	Kenya	0.06437	34.66981	1548
38-K	Kenya	-0.515672	37.36573	1398
39-K	Kenya	0.093449	37.81905	1155
40-K	Kenya	0.758926	34.87193	1680
41-K	Kenya	-0.78523	36.91462	1928
42-K	Kenya	-0.080452	34.82601	1150
43-K	Kenya	-1.136357	34.60511	1389
44-K	Kenya	0.143038	34.62155	1430
45-K	Kenya	0.091232	37.81236	1164
46-K	Kenya	0.274213	34.59082	1310
47-K	Kenya	-0.506081	37.12583	1706
48-K	Kenya	0.092842	37.82547	1160
49-K	Kenya	-0.985336	36.94538	1625
50-K	Kenya	0.77217	34.81198	1635
51-K	Kenya	-0.965147	34.48188	1393
52-K	Kenya	0.331085	34.3008	1269
53-K	Kenya	0.756035	34.85681	1651
54-K	Kenya	-0.783291	36.93494	1751
55-K	Kenya	1.278913	35.09086	2075

Farmer/Sample ID	Country	Latitude	Longitude	Altitude
56-K	Kenya	0.784362	34.86308	1685
57-K	Kenya	0.771604	34.78512	1689
01-M	Malawi	-14.19613	33.76749	1182
02-M	Malawi	-14.21196	33.75928	1173
03-M	Malawi	-14.1534	33.78207	1108
04-M	Malawi	-14.18489	33.77386	1131
05-M	Malawi	-14.20842	33.74425	1147
06.M	Malawi	-14.19637	33.77437	1182
07-M	Malawi	-14.19637	33.77437	1146
08-M	Malawi	-14.23192	33.80106	1185
09-M	Malawi	-14.19613	33.76749	1182
10-M	Malawi	-14.16169	33.83935	1124
11-M	Malawi	-14.20348	33.78574	1166
12-M	Malawi	-14.21442	33.76104	1182
13-M	Malawi	-14.16032	33.8459	1123
14-M	Malawi	-14.19999	33.80179	1164
15-M	Malawi	-14.21487	33.8955	1146
16-M	Malawi	-14.2108	33.8244	1173
17-M	Malawi	-14.06896	33.81357	1085

Farmer/Sample ID	Country	Latitude	Longitude	Altitude
18-M	Malawi	-14.19637	33.77437	1182
19-M	Malawi	-14.21487	33.8955	1146
20-M	Malawi	-14.1431	33.84702	1107
01-TZ	Tanzania	-3.40427	36.79448	1174.3
02-TZ	Tanzania	-3.40427	36.79447	1183.9
03-TZ	Tanzania	-8.0209	35.8564	1883.3
04-TZ	Tanzania	-7.83219	35.84432	1677
05-TZ	Tanzania	-4.137065	37.9056	1399
06-TZ	Tanzania	-3.2511	37.24033	1254.9
07-TZ	Tanzania	-4.135077	37.91367	1181
08-TZ	Tanzania	-3.26394	37.35318	1289.3
09-TZ	Tanzania	-8.920116	33.52334	1788
10-TZ	Tanzania	-9.232094	33.63924	1448
11-TZ	Tanzania	-6.83867	37.74604	764.57
12-TZ	Tanzania	-6.84718	37.69085	899
13-TZ	Tanzania	-4.142703	37.90506	1428
14-TZ	Tanzania	-4.136123	37.91188	1202

Appendix 2: Effects of Foliar Fertilizer, Synthetic Pesticides and Botanical Plants Extract on Common Beans Growth

Treatments	Plant height (cm)	Number of leaves	Number of branches	Leaf area	Stem width(mm)	Leaf greenness
Treatment applied						
Foliar fertilizer	36.71±1.17 ab	3.13± 0.13ab	2.94±0.21 bc	21.47±0.38 ab	3.72±0.08 a	3.28±0.21 b
Synthetic Pesticide	33.22±1.20 c	2.75± 0.17c	2.69± 0.22bc	20.49±0.49 b	3.37 ±0.09b	3.38 ±0.27b
T. vogelii	39.63±1.38 a	3.25±0.17 a	3.63± 0.18a	22.68± 0.58a	3.73±0.10 a	4.81±0.13 a
T. diversifolia	36.48±0.89 b	3.25± 0.14a	3.06±0.11 b	21.68±0.72ab	3.77± 0.10a	4.56±0.16 a
Water	34.11±0.88 bc	3.00±0.00abc	2.50± 0.13c	21.57±6.0.37ab	3.31 ±0.07b	3.09± 0.19b
Water and Soap	36.24±1.20 bc	2.88± 0.09bc	2.69± 0.12bc	21.33±0.71ab	3.43±0.08 b	3.34 ±0.25b
Method of application						
Foliar spray	35.80 ±0.81a	3.00±0.07 a	3.02± 0.12a	21.95±0.36 a	3.52 ±0.06a	3.79± 0.17a
Soil drench	36.33 ±0.58a	3.08± 0.09a	2.81± 0.09a	21.09±0.28 b	3.59 ±0.05a	3.70±0.14 a
2 way ANOVA (F statistics)						
Treatment	4.26**	2.38*	5.56***	1.86**	5.70***	12.36***
Method of application	0.36ns	0.60ns	2.26ns	4.16*	1.16ns	0.30ns
Treatment*Method of application	2.71*	0.60ns	0.31ns	3.52**	1.00ns	0.89ns

The values presented are means ± SE. *, **, * =significant at P ≤ 0.05, P ≤ 0.01, P ≤ 0.001 respectively, ns=not significant. Means followed by similar letter (s) in a column are not significantly different**

Appendix 3: Correlation Matrix (Pearson (n) Showing the Association between Bean Plant Growth, Yield Parameters and Common Bean Metabolites

Variables	CC	FL	AC	PH	NL	NB	LA	STW	LG	NPP	SY	Phen.	Trypt.	Ru.
CC	**1**													
FL	**0.715**	**1**												
AC	0.013	-0.038	**1**											
PH	**0.772**	0.452	0.188	**1**										
NL	**0.690**	**0.682**	0.489	**0.707**	**1**									
NB	**0.936**	0.575	-0.014	**0.654**	**0.608**	**1**								
LA	**0.597**	0.133	0.186	**0.683**	0.374	0.560	**1**							
STW	**0.718**	**0.691**	-0.050	**0.668**	**0.738**	**0.620**	0.251	**1**						
LG	**0.754**	0.483	-0.039	**0.652**	**0.604**	**0.815**	**0.583**	**0.661**	**1**					
NPP	**0.942**	**0.678**	-0.012	**0.716**	**0.687**	**0.887**	**0.689**	**0.671**	**0.781**	**1**				
SY	**0.956**	**0.738**	0.070	**0.781**	**0.784**	**0.884**	**0.653**	**0.762**	**0.814**	**0.938**	**1**			
Phen.	0.559	0.461	-0.257	0.131	0.022	**0.583**	0.323	0.151	0.402	0.515	0.471	**1**		
Trypt.	0.574	0.379	-0.141	0.167	0.066	**0.631**	0.375	0.132	0.425	0.527	0.478	**0.980**	**1**	
Ru.	0.548	0.353	-0.302	0.158	0.088	**0.608**	0.201	0.250	0.272	0.471	0.451	**0.815**	**0.846**	**1**
Values in bold are different from 0 with a significance level alpha=0.05														

Appendix 3: Eigenvalues of the Principal Component Analysis

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Eigenvalue	8.032	2.560	1.199	0.915	0.382	0.362	0.222	0.163	0.095	0.047	0.024
Variability (%)	57.374	18.286	8.566	6.535	2.727	2.587	1.585	1.161	0.677	0.332	0.171
Cumulative %	57.374	75.660	84.226	90.761	93.487	96.074	97.659	98.820	99.497	99.829	100.000

Appendix 4: Factor Loadings and Correlations between Variables and Factors CC = Chlorophyll content; FL = Flavonoids; AN = Anthocyanins; PH = Plant height; NL = Number of leaves; NB = Number of branches; LA = Leaf area; SW = Stem width; LG = Leaf greenness; NPP = Number of pods per plant; SY = Seed yield/plant; Phen=Phenylalanine; Trypt = Tryptophan and Ru=Rutin

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
CC	0.975	-0.007	-0.019	-0.005	-0.089	0.011	-0.102	0.114	0.116	-0.029	-0.056
FL	0.732	-0.045	-0.494	0.301	0.068	0.340	0.013	0.041	-0.047	-0.025	0.052
AC	0.024	-0.562	0.493	0.650	0.036	-0.096	0.049	-0.005	0.069	-0.006	-0.002
PH	0.768	-0.418	0.151	-0.201	-0.281	0.035	0.197	0.220	-0.056	0.040	-0.002
NL	0.726	-0.581	-0.100	0.307	0.006	-0.058	-0.042	-0.064	-0.141	0.041	0.000
NB	0.932	0.097	0.051	-0.037	0.053	-0.223	-0.188	0.128	0.050	-0.038	0.092
LA	0.640	-0.113	0.643	-0.291	-0.056	0.208	0.024	-0.169	-0.011	-0.038	0.052
STW	0.744	-0.325	-0.457	-0.088	-0.084	-0.151	0.204	-0.173	0.148	0.005	0.021
LG	0.831	-0.113	0.037	-0.266	0.411	-0.190	0.107	0.016	-0.089	0.007	-0.017
NPP	0.951	-0.039	0.040	-0.090	0.020	0.114	-0.202	-0.076	0.048	0.145	-0.025
SY	0.974	-0.137	-0.018	-0.019	0.001	0.054	-0.061	-0.054	-0.028	-0.127	-0.073
Phen.	0.597	0.749	0.084	0.165	0.102	0.124	0.141	0.021	0.037	0.001	-0.006
Trypt.	0.614	0.718	0.206	0.209	0.072	-0.012	0.118	0.019	0.024	0.040	-0.008
Ru.	0.565	0.701	-0.041	0.151	-0.301	-0.221	-0.031	-0.102	-0.117	-0.006	0.001

Appendix 5: Squared Cosines of the Variables of the Principal Component Analysis: CC = Chlorophyll content; FL = Flavonoids; AN = Anthocyanins; PH = Plant height; NL = Number of leaves; NB = Number of branches; LA = Leaf area; SW = Stem width; LG = Leaf greenness; NPP = Number of pods per plant; SY = Seed yield/plant; Phen = Phenylalanine; Trypt = Tryptophan and RU- Rutin

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
CC	**0.951**	0.000	0.000	0.000	0.008	0.000	0.010	0.013	0.014	0.001	0.003
FL	**0.535**	0.002	0.244	0.091	0.005	0.116	0.000	0.002	0.002	0.001	0.003
AC	0.001	0.316	0.243	**0.423**	0.001	0.009	0.002	0.000	0.005	0.000	0.000
PH	**0.589**	0.175	0.023	0.040	0.079	0.001	0.039	0.048	0.003	0.002	0.000
NL	**0.527**	0.338	0.010	0.094	0.000	0.003	0.002	0.004	0.020	0.002	0.000
NB	**0.869**	0.010	0.003	0.001	0.003	0.050	0.035	0.016	0.003	0.001	0.009
LA	0.410	0.013	**0.414**	0.084	0.003	0.043	0.001	0.029	0.000	0.001	0.003
STW	**0.554**	0.106	0.209	0.008	0.007	0.023	0.042	0.030	0.022	0.000	0.000
LG	**0.690**	0.013	0.001	0.071	0.169	0.036	0.012	0.000	0.008	0.000	0.000
NPP	**0.905**	0.002	0.002	0.008	0.000	0.013	0.041	0.006	0.002	0.021	0.001
SY	**0.949**	0.019	0.000	0.000	0.000	0.003	0.004	0.003	0.001	0.016	0.005
Phen.	0.357	**0.561**	0.007	0.027	0.010	0.015	0.020	0.000	0.001	0.000	0.000
Trypt.	0.377	**0.515**	0.042	0.044	0.005	0.000	0.014	0.000	0.001	0.002	0.000
Ru.	0.319	**0.492**	0.002	0.023	0.091	0.049	0.001	0.010	0.014	0.000	0.000

Values in bold correspond for each variable to the factor for which the squared cosine is the largest

Appendix 6: Squared Cosines of the Observations: FS= Foliar Spray, SD=Soil Drench

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Foliar fertilizer_FS	**0.697**	0.008	0.009	0.105	0.088	0.092	0.000	0.000	0.001	0.000	0.000
Foliar fertilizer_SD	**0.352**	0.184	0.239	0.101	0.019	0.009	0.037	0.035	0.021	0.000	0.002
Synthetic _FS	0.123	**0.843**	0.001	0.017	0.006	0.004	0.000	0.000	0.004	0.001	0.000
Synthetic _SD	**0.598**	0.014	0.135	0.162	0.000	0.033	0.001	0.008	0.026	0.023	0.001
T. diversifolia _FS	**0.692**	0.003	0.032	0.077	0.099	0.061	0.012	0.023	0.000	0.001	0.000
T. diversifolia _SD	0.118	0.114	**0.409**	0.263	0.034	0.038	0.014	0.001	0.007	0.001	0.000
T. vogelii _FS	**0.891**	0.029	0.014	0.021	0.010	0.031	0.001	0.001	0.002	0.000	0.000
T. vogelii _SD	**0.674**	0.174	0.009	0.000	0.039	0.001	0.021	0.070	0.011	0.001	0.000
Water _FS	**0.669**	0.003	0.134	0.080	0.007	0.004	0.085	0.006	0.012	0.001	0.000
Water _SD	**0.596**	0.212	0.115	0.020	0.002	0.003	0.031	0.003	0.009	0.002	0.005
Water and soap_FS	**0.702**	0.076	0.057	0.099	0.007	0.001	0.002	0.022	0.000	0.031	0.003
Water and soap_SD	**0.443**	0.166	0.266	0.004	0.036	0.015	0.032	0.009	0.008	0.001	0.018

Values in bold correspond for each observation to the factor for which the squared cosine is the largest

Appendix 7: Survey of Farmers' Awareness on the Use of *T. vogelii*

A kobo toolbox link for the questionnaire survey that evaluated perception about use of *T. vogelii* (https://ee.kobotoolbox.org/x/#nXf5tx5B).

Appendix 8: Survey of Farmers' Awareness on the Use of *T. vogelii*

A kobo toolbox link for the questionnaire survey that evaluated perception about use of *T. vogelii* (https://ee.kobotoolbox.org/x/#nXf5tx5B).