INFLUENCE OF PLANT METABOLITES ON FLEA BEETLE, *Phyllotreta mashonana* (Coleoptera, Chrysomelidae) DAMAGE ON SPIDER PLANT, *Cleome gynandra*. L. MORPHOTYPES

BY

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DECLARATION

Declaration by the Candidate.

This thesis is my original work and has not been presented for a degree in any other University. No part of this thesis may be reproduced without the prior consent of the author or University of Eldoret.

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DEDICATION

This work is dedicated to my beloved family for their invaluable input.

ABSTRACT

Spider plant (*Cleome gynandra*.L) is an African Leafy Vegetable (ALV). The crop is well adapted to a wide range of ecologies and has been differentiated into three main morphotypes based on the stem colours of purple, purple-green and green stemmed morphotypes. The plant plays an important role as a source of nutrients, income and traditional medicines in Kenya. However, production of spider plant in Kenya has been constrained by poor agronomic practices, plant genotypes which are less productive and pests. Flea beetle (*Phyllotreta mashonana*) is an important pest which accounts for 25 % foliage damage in the plant manifested as "shot holes.'' The reported damage however, was variable among the morphotypes. The study aimed at determining the level of damage inflicted on the three main spider plant morphotypes in relation to the nutritive value and phytochemicals of importance for plant defense and/or pest attraction. The research entailed propagation of three spider plant morphotypes inside a screen house in a C.R.D. Flea beetles were introduced after seven weeks of growth to determine the damage caused by the beetles on the leaves which was assessed using the Bailey scoring scale of 0-3, 4-7 and>7 holes per leaf. The data on damage inflicted by the flea beetle was log transformed to standardize the variance and then analysed using ANOVA. The means were separated using L.S.D 0.05. It was observed that, the purple stemmed morphotype was the most damaged with 8.6 holes $p<0.05$, while the green stemmed morphotype was least damaged with 2.7 holes $p<0.05$. Quantitative determination of nutrients and secondary metabolites was done on the foliage taken on the 7th week of growth. Proximate test was done to determine the carbohydrates and proteins were determined by Kjedahl method. Determination of the secondary metabolites and vitamins was done using the High Power Liquid Chromatography (HPLC). There was a significant difference $p<0.05$ of the primary and secondary metabolites among the three morphotypes with purple stemmed having the highest quantities of carbohydrates, proteins, vitamins and glucosinolates. The purple morphotype had the least terpenoid quantities. In contrast, the green morphotype had the least primary metabolites and glucosinolates with the highest quantities of terpenoids. Correlation analysis showed a positive relationship of primary metabolites and glucosinolates with the flea beetle damage, while a negative correlation was noted between the terpenoids and the flea beetle damage $r=-0.794$. From this study, it was concluded that, the purple stemmed morphotype was the most susceptible to the flea beetle damage and green stemmed was the least susceptible. In addition, it emerged that the inflicted damage in spider plant morphotypes by the flea beetle is largely metabolomically influenced and that primary metabolites and glucosinolate levels are antagonistic to the terpenoids levels in the morphotypes, so do their flea beetle resistance roles. It can therefore be recommended that, plant breeders can use the green stemmed morphotype to improve the resistance of the more nutritious but flea beetle susceptible purple stemmed morphotype in order to increase the yields and maintain the nutritional potential of the morphotypes.

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LIST OF ABBREVIATIONS AND ACRONYMS

ALVs-African Leafy Vegetables

ANOVA-Analysis of variance

ATP-Adenosine tri-phosphate

CRD-Completely Randomised Design

DAI-Days after infestation

HPLC-High powered liquid chromatography

IPGRI-International plant genetic resources institute

JKUAT-Jomo Kenyatta University of Agriculture and Technology

KFSSG-Kenya Food Security Steering Group

P-Purple stemmed spider plant morphotype

P-G-Purple-Green stemmed spider plant morphotype.

G-Green stemmed spider plant morphotype.

UV-Ultra violet

WUE-Water use efficiency

TCA-Trichloroacetic acid

CHAPTER 1

INTRODUCTION

African Leafy Vegetables (ALVs) are important sources of food and income in many parts of the world. The vegetables are well adapted to a wide range of ecologies due to their long evolutionary history (Nekesa and Meso, 2005). The leaves of the ALVs are often used as vegetables and also as a source of income for the rural people mainly women. There is a large number of species that are used as ALVs, in Kenya however, amaranths, solanums, cucurbits, slender leaf, spider plant, cow pea and jute mallow are the most predominant (Table.1).

Table.1 Percentage (%) Consumption of ALVs by Western Kenya communities.

Common name	Scientific name	% consumption
Cowpeas	*Vigna unguiculata*	30
Leaf amaranths	*Amaranthus blitum*	21
African nightshades	*Solanum villosum*	12
Jute mallow	*Corchorus olitorius*	11
Spider plant	*Cleome gynandra*	7
Slender leaf`	*Crotalaria brevidens*	7
African kale	*Brassica oleracea*	7
Pumpkin leaves	*Curcubita carinata*	5
Total		100

(Source: Abukutsa-Onyango, 2004)

In the predominantly carbohydrate-based diets of rural Kenyans; proteins, vitamins and minerals found cheaply in green leafy vegetables are often lacking and the alternative sources i.e. animal and legumes are out of reach to most rural families because of their prohibitive prices. These nutrients and minerals are found in significant quantities in the ALVs (Table 2.) thus can offer a solution to the existing malnutrition and health problems (KFSSG, 2009).

African Leafy Vegetables are easy and fast to grow therefore important in filling the gap in food production (Kimiywe *et al.*, 2007). Unlike the exotic vegetables that require high input than most rural households can afford, ALVs do not require high inputs, are superior nutritionally and there is the indigenous knowledge on how to grow them (KENRIK, 2004) This indigenous knowledge however, requires scientific enhancement to contribute towards higher agro-biodiversity in crop systems, which is not only desirable from a conservation point of view, but increases food security at the house hold level (Chigwumire *et al.,* 1998; Onyango, 2001).

According to Kenya Food Security Steering Group KFSSG (2009), food security in Kenya has declined at an alarming rate of 28 % for the last one decade; this has been attributed to the climatic changes, poor agronomic practices and urbanization. This trend can only be reversed by production of the already adapted crops which do not require large sizes of land, making ALVs one of the ideal crops for production.

Table 2. Comparative nutritional and mineral composition of ALVs and exotic vegetables (in % or mg/100 g edible parts.)

Nutrient	Spider plant	Amaranth	Nightshade	Cabbage	Kale
Moisture content (%)	81.8-89.6	84	87.2	91.4	88.2
Crude protein (%)	3.1-7.7	4.6	4.3	1.7	2.3
Crude fibre (%)	1.3-1.4	1.8	1.3	1.2	1.2
Carbohydrates (%)	4.4-6.4	8.2	5.7	6.0	5.9
Potassium (mg)	410				
Calcium (mg)	213-434	410	442	47	189
Magnesium (mg)	86				
Sodium (mg)	33.6				
Phosphorous (mg)	110-115	103	75	40	91
Iron (mg)	1-11	8.9	1.0	0.7	4.8
Zinc (mg)	0.76				
Copper (mg)	0.46				
B-carotene (mg)	10,400-10,500	5,716	3660	100	800
Ascorbic acid (mg)	12-18	64	20	54	58
Total phenolics (mg)	520-910				

(Source: Kenya Resource Centre for Indigenous Knowledge (KENRIK, 2004).

Spider plant is widely grown in Kenya, but it is predominant in Western, Rift Valley and Coastal regions. In western Kenya particularly, the plant contributes 7% of total ALVs used, thus becoming an important food crop in these areas (Omondi, 1989; Abukutsa-Onyango, 2003; Abukutsa-Onyango, 2004). The plant is preferred due to its drought tolerant nature attributed to its deep tap root and water utilization efficiency (Mnzava, 1999; Waswa *et al.*, 1996). The plant being a C4 species has a high photosynthesis efficiency characterized by rapid growth and high dry matter production i.e. 3-5 times more per unit leaf area and unit time than C3 plants. This can partly be attributed to the diaheliotropic leaf movements ensuring maximum exposure to sunlight for maximum photosynthesis. This is a mechanism commonly found in plants that need to complete their cycle rapidly before the onset of drought thus the preference as a drought tolerant crop (Imbamba, *et al*., 1977; Rao and Rajendrudu, 1999).

Spider plant has been differentiated into 3 main morphotypes based on the stem colour i.e. the purple, purple-green and green stemmed (Plate 1). According to K'Opondo (2009; 2011) and spider plant morphotypes vary in many other aspects including; the plant structure, stem pubescence, days to 50 % flowering, plant height, petiole length and fruit breadth. In addition, when the morphotypes were subjected to dendrogram analysis, there emerged three clusters of different morphotypes clearly showing that the morphotypes are unique.

Apart from the stated morphological differences between the spider plant morphotypes, attraction and repulsion to natural enemies or insect herbivores also vary between the

morphotypes (Abukutsa-Onyango, 2009). This has been attributed to the ubiquitous organic compounds produced by the morphotypes. These compounds include the glucosinolates which forms an important part of the pest attractants especially in the Cruciferous and Capparaceae flea beetles. Glucosinolates have been found to initiate and prolong feeding of the flea beetles in Canola (Addesso, 2009). Spider plant, being a member of the Capparaceae family which is related to the Brassicaceae family is therefore thought to have these glucosinolates in significant quantities serving as attractants to the flea beetles which cause damage on the plant foliage while feeding (Bartlet, 1996). On the other hand, terpenoids form important anti-herbivory compounds. Plants with high levels of terpenoids like the glabrous type of Canola have been observed to repel pests. Most cruciferous plants have also been observed to contain substantive levels of terpenoids which form part of host plant resistance to herbivorous pests in these plants (Feeny *et al*., 1989).

Analysis of metabolites in relation to spider plant morphotypes and the inflicted damage by the flea beetles forms an important basis in understanding the relationship of metabolites and flea beetle infestation.

1.1 PROBLEM STATEMENT.

Spider plant morphotypes have been differentiated morphologically and dendrogramatically into 3 main morphotypes. There are other emerging differences which have not been documented, for instance, Abukutsa-Onyango (2009) in her analysis of the spider plant morphotypes described the purple stemmed morphotype as being more nutritious and susceptible to diseases than the other morphotypes. In addition to

Abukutsa-Onyango's report, many farmers in Western and Rift Valley provinces have found out that the spider plant morphotypes have exhibited variable damage caused by the flea beetles.

Plate.1a Purple stemmed morphotype

(Source: Author, 2013)

Plate. 1b Purple-green stemmed morphotype

(Source: Author, 2013)

Plate.1c Green stemmed morphotype.

(Source: Author, 2013)

According to Cassareto and Corcuera (1998), flea beetles just like other insects require to ingest a given amount of food for optimum development. The level of feeding on a particular plant species is determined by the quantity and proportion of nutrients available for ingestion. The pest therefore feeds more on the highly nutritious species when there is

a variety of species with different quantities of nutrients at their disposal in order to gain the maximum nutrient benefit. The overall nutritional profile of spider plant has been done but specific information on the nutritional level per morphotype has not been documented. In addition to the nutritional aspect, the level of secondary metabolites per spider plant morphotype has not also been documented. The study anticipated that just like the morphotypes varied in the morphological and dendrogramatical characteristics, the metabolites i.e. primary and secondary also varied.

Attributing the variation of damage by the flea beetles to the metabolites was informed by the fact that metabolomics play a great role in host plant desirability by the pests. This is due to the fact that, metabolites play the attractant or repellent role in form of terpenoids which are produced in variable quantities by plants while other metabolites act as feeding stimulants in form of glucosinolates and primary metabolites.

The study investigated the primary metabolites, secondary metabolites and flea beetle damage caused per spider plant morphotype as this has not been documented.

1.2 JUSTIFICATION

Spider plant forms a significant part of the most consumed ALVs in Western and Rift Valley provinces of Kenya. It forms 7 % of the total ALVs consumption in these regions. The crop is preferred due to its high nutritional profile, therapeutic role and ease of production. The crop has also been ingrained in the culture of these communities forming an integral dietary component for these communities. Due to this significant role to these communities, any inimical condition to the production of the crop is a matter of concern.

Spider plant is well adapted to these regions although some pests have been found to attack it and reduce the yield. Among the major pests include; flea beetles, bragada bug, aphids and nematodes. However, it is the flea beetle infestation that has been reported to cause the greatest damage (Mnzava and Chigumira, 2004). The pest if left unabated and in severe infestation can result in total crop failure while moderate infestation leads to at least 25 % foliage damage characterized by "shot holes" on the foliage.

Determination of the actual damage caused by the flea beetles per morphotype was necessary in documentation and scientific validation of the indigenous communities' observation of the Western and Rift Valley provinces of Kenya that the damage varies across the morphotypes. The study investigated the variation existing between the spider plant morphotypes in terms of flea beetle damage and metabolites thereby offering a scientific explanation to the role of metabolites on flea beetle damage variation in spider plant morphotypes. The research aimed at generating important information on increasing the quality and quantity of spider plant yield by utilizing the inherent host-plant resistance attributes of the morphotypes in order to make cultivation of the crop attractive to more farmers and the product fetch higher prices in the market due to improved yield and quality respectively.

1.3 OBJECTIVES

Main objective

The overall objective of the study was to enhance production of spider plant in western Kenya by metabolomics.

Specific Objectives

The specific objectives of the study were to;

1. Determine the preference and damage of the three spider plant morphotypes by flea beetle.

2. Determine the plant metabolites composition of the spider plant morphotypes.

3. Correlate flea beetle damage on the spider plant morphotypes with the secondary metabolites and primary metabolites.

1.4 Hypothesis

Ho: There is no difference in flea beetle damage between the three spider plant morphotypes.

Ho: There is no significant difference in primary and secondary metabolites among the three spider plant morphotypes.

Ho: There is no correlation between primary metabolites, secondary metabolites and flea beetle damage in the three spider plant morphotypes.

CHAPTER 2

LITERATURE REVIEW

2.1 Spider plant (*Cleome gynandra*.L).

Spider plant belongs to the Genus *Cleome* and the Family Cleomoideae. Some of the common names include; African cabbage, Spider flower, Spider wisp and Cat's whiskers. The plant is a primitive relative of the Brassicas cultivated mainly as an African leafy vegetable (ALV) (Schippers, 2002; Chweya, 1997). These families contain secondary metabolites i.e. glucosinolates and terpenoids that give them the characteristic taste and smell respectively (Rodman *et al.,* 1997; Tentelier, 2007).

The *Cleome* genus comprises 150-200 species, with about 50 of them occurring in Africa (Mnzava and Chigumira, 2004). Spider plant is an erect annual herb reaching up to 1.5 m tall, strongly branched with long tap root and a few secondary roots, the stem is hairy and rather oily, the leaves are alternate and palmately compound with 3, 5 and 7 leaflets borne on petioles 2-10 cm long (Schippers, 2002 ; Mnzava and Chigumira, 2004; AVRDC, 2000).

Spider plant is both self and cross pollinated and bears white or pink flowers on a long much branched inflorescence resulting in fruits which later dehisce to release small, rough, and grey to black seeds (Maundu *et al*., 1996).

In Kenya, it is known by different names among the different communities. Among the Swahili (Mwangani), Luhya (Tsisaka), Luo (Dek), Kalenjin (Saget), Kisii (Chinsanga), Kikuyu (Thageti) and Kamba (Mwianzo) (Chweya, 1997).

The leaves and seeds of spider plant are used in indigenous medicine in many countries. These medicinal uses include; sap from leaves used as analgesic, concoction of boiled leaves and/or roots administered to facilitate child birth, stomachache reliever, deworming, treatment of conjunctivitis, chest pain reliever, anti-inflammatory proteins, treatment of anemia, wounds and other disorders (Mallikharjuna *et al.,* 2007; Abukutsa-Onyango, 2004; Opole *et al.,* 1995). The plant has significant economic contribution mainly to the rural women with 0.7% of the vegetable revenue earned from the sale of spider plant foliage in the rural and urban areas (Maundu., 1997).

2.1.1 Spider plant production constraints

The production of spider plant in Kenya has been constrained by factors such as short vegetative stage, lack of certified seed, insect pests and weeds. Important insect pests include; flea beetles, aphids, bragada bug and ants. The pests lower both the qualitative and quantitative value of the crop.

In severe infestation, flea beetles cause up to 25 % foliage loss, though when infestation occurs at a tender stage this may lead to total crop failure (Maundu, 1997; Mbugua *et al.,* 2005).

2.2 Flea beetle damage in spider plant

2.2.1 Biology of the flea beetle

Flea beetles belong to the Family Coleoptera, Sub Family Alticinae (derived from the Greek “haltikos” meaning “good at jumping”) the pests are minute oval shaped beetles whose enlarged hind femora are adapted to jumping over long distances a habit that have earned them the name flea beetles. (Plate. 2)

Plate. 2 Flea beetle *Phyllotreta mashonana* (mg x20)

(Source: Mc Caffrey, 2004)

Many species of the beetles exist, and each species is adapted to locate, feed and produce on a certain plant group (Chitteden and Marsh, 1990; Bycznski, 1999). A case in point is the crucifer flea beetle (*P. cruciferae*) which feeds on Brassica crops while *P. mashonana* mainly attacks the *Cleome* genus. The two species resemble each other but can be distinguished by their size and colour. The crucifer flea beetle has two yellow stripes on its back while *P. mashonana* has shiny black elytra and is smaller in size (Mc Caffrey, 2004).

2.2.2 Flea beetle damage in plants

The most important damage of these univoltine species is caused by the adult flea beetles. Flea beetles cause sieve-shaped damage known as “shot holes” on the plant foliage which upsets the water balance in the plant tissues. They cause high level of mortality of seedlings, growing in young plants is retarded and they cause unequal ripening of fruits (Giamoustaris and Mithen, 1995). The importance of *Phyllotreta* species as pests is aggravated by the fact that several species such as the corn flea beetle are known to act as

vectors of numerous plant pathogens. In this case, the corn flea beetle harbours the Stewart's wilt bacterium and spreads the disease during the growing season. The bacterium incubates in the digestive system of the beetle and 10-20% of the corn beetles pass it onto their hosts during feeding. An uninfected beetle that feeds on infected plant can spread the bacterium to other plants (Wety, 2007; De Jong *et al*., 2000).

In canola, adult flea beetles begin feeding as soon as seedlings emerge. Damage by flea beetle to the seedlings can be severe and plants may lose up to 100% of their leaf area, the flea beetles inflict holes of approximately 1mm in diameter on the leaf surface. Feeding by the adult beetles on cotyledons and first true leaves is easily quantified by counting the number of pits per leaf and employing leaf size as a covariate (Bailey, (1996). Pilson, (2007) has also shown that feeding by adult flea beetles on seedlings can significantly reduce numbers and mass of seeds produced by *Brassica rapa.* Flea beetles can also significantly reduce canola seed yields (Mc Caffrey, 2004). Flea beetles oviposit in the soil and at the base of their host plants and larvae feed on host plant roots; root damage is generally not severe though when the population is high, it can cause massive seedlings failure at the tender stage (Cook, 2003).

2.2.3 Pest status of *Phyllotreta mashonana*

The adult lays yellow tiny and elongated eggs. The larvae are dirty white in colour with brown heads. When mature, they are slender up to 6 mm long with 3 pairs of short legs on the thorax, the pupae are white and about 2-4 mm long. The adults are small and blackish beetles with a metallic or bright blue lustre, measuring about 2-3 mm length (Peterson *et al.,* 2000).

Beetles of the first generation are the most destructive to plants particularly during the dry season. Adult flea beetles feed on the foliage of plants and because of their small size and active habits they eat little at each feeding site producing damage consisting of small round holes often described as "shot holes", when the feeding is prolonged, the leaves tend to appear like a perforated net (Raubenheimer and Simpson, 1993; Cosse *et al.,* 2006).

2.2.4 Host plant-beetle interaction

Flea beetle is a polyphagous insect with marked preference to the Brassicas and all related families like the Capparaceae and Solanaceae. The host plants are usually colonized by the jumping beetles which lands on the plants and through probing with its mouth adapted for biting and chewing aided by the olfactory, visual and organoleptic cues are able to determine the right host (Thompson, 2005).

This initial investigation on the surface of the plant involves little or no biting, but enables the beetle to sense the host suitability within a very short time.

2.3 Flea beetle damage and metabolites

Metabolites are substances produced by plants during metabolism and are defined as primary metabolites if they are directly involved in the normal growth, development and reproduction e.g. carbohydrates, proteins and vitamins or secondary metabolites if they are not directly involved in the normal processes but have an important ecological function e.g. terpenoids, glucosinolates and alkaloids. The quantitative measurement of the dynamic metabolic response of living systems to pathophysiological stimuli or

genetic modification is known as metabolomics (Spencter, 2009; Hall, 2006; Cook, 2008).

Metabolomics has attracted significant interest over the last few years and has contributed significantly to the discovery of bioactive compounds, improving food quality and cataloguing metabolism. It is now being used as a new diagnostic and therapeutic approach to study plant-herbivore interactions (Dixon and Payne, 1980; Boulter, 2008; Jansen *et al.*, 2009).

According to Shinoda *et al.*, (2003); Michael and Boyko (2006) the ability of crops to accumulate low molecular weight bioactive compounds oftenly known as metabolites provides a chemical defense against herbivorous insects. On the other hand, some metabolites play a major role in host plant selection due to their ubiquitous characteristics.

Nielsen *et al*., 2001; Agerbirk *et al.,* (2001); Nielsen (2005) found out that the interaction between *Barbae vulgaris* (Brassicaceae) and the flea beetles *P. nemorum* and *P. cruciferae* is a unique model system with the plant showing polymorphic behaviour with respect to insect resistance, the pubescent (P)-type is susceptible to all known flea beetle species, whereas the glabrous (G)-type is resistant to most common species of the insects.

The (P) and (G)-type morphotypes of *B. vulgaris* differ morphologically, biochemically and cytologically. Of interest is that, there is significant difference in the levels of glucosinolates with the (P)-type having the highest quantities of glucosinolates. These

metabolites can be attributed to the flea beetle resistance/susceptibility observed within the spider plant morphotypes (Verma and Pandey, 1998; Kuzina *et al*., 2009).

2.3.1 Flea beetle damage and glucosinolates

Glucosinolates are low molecular mass nitrogen and sulfur-containing compounds that are hyhrolysed by myrosinase. Glucosinolates occur as secondary metabolites in almost all plants of the order Brassicales including the families Brassicaceae, Capparidaceae and Caricacaceae. They are feeding and oviposition stimulants for a number of specialist insects, which have become adapted to such compounds as an outcome of long-standing co-evolutionary interactions with host plants containing them (Bartlet *et al*., 2011; Finch, 1980; Renwick, 2002; Thompson, 2005). Though, the relationship between glucosinolate profiles of plants is not simple, the (P)-type *B. vulgaris* contains the R-isomer of 2-hydrox-phenylethyl glucosinolate, whereas the (G)-type contains in S-isomer. The two morphotypes showed significant difference in their resistance to flea beetle damage (Agerbirk *et al.,* 2003). These glucosinolates are responsible for the variation of damage that exists between the two varieties of canola as the P-type with higher quantities of glucosinolates attracted more feeding insects thus more damage than the G-type which had a low quantity of the glucosinolates. Spider plant being a Capparaceae family member is known to contain significant quantities of glucosinolates; these compounds however, just like in other plants may vary from morphotype to morphotype and season to season therefore the variation in flea beetle attraction and the associated damage in the plant.

2.3.2 Flea beetle damage and terpernoids

Terpenoids are aromatic lipids derived from 5-carbon isoprene units with a major role in anti-macrobial, anti-fungal, antineoplastic, insecticidal, molluscicidal, and other pharmaceutical functions. They are widely distributed in higher plants and are constituents of many plant drugs and folk medicines (Fische, 1991; Sparg *et al*., 2004, Gauthier *et al.,* 2009).

The toxicity of terpenoids to fungi and insects is thought to be as a result of their ability to form complexes with sterols in the plasma membrane thus destroying the cellular semi permeability leading to cell death. Although terpenoids are toxic to poikilotherms, their oral toxicity to mammals is low. This therefore, makes terpenoids ideal pest damage antidote as they induce inherent resistance without associated diet complications to humans (Mazza, 2007).

These terpenoids have been developed as a putative response to renewed selection pressure from herbivorous insects, a number of crucifers and related families have evolved a second generation of secondary defense to pests (Renwick, 2002).

The impact of terpenoids in plants on the flea beetle defense cannot be overemphasized, for instance in *B. vulgaris* the glabrous (G)-type, concentration of terpenoids was found to correlate positively with flea beetle resistance. In the plant, the terpenoids levels declined during the dry spell where, there was a notable decrease in the resistance to the flea beetle during the same period (Agerbirk, 2011; Agerbirk *et al.*, 2003). This spatial fluctuations of terpenoids even within the same plant resulted in variable flea beetle resistance. This therefore confirms that, terpenoids are feeding deterrents for the flea beetles (Shinoda *et al.*, 2003; Agerbirk *et al.,* 2003).

2.3.3 Flea beetle damage and primary metabolites.

Host plant susceptibility or resistance is however, not solely mediated by secondary plant metabolites as plant damage by the phytophagous insects correlates better with nutrients levels than with glucosinolate levels (Louda and Mole, 1991; Mitchell, 1999; Hiiesaar *et al.*, 2006).

According to Beran (2011), Sugars, amino acids, proteins, vitamins, sterols and phospholipids have all been reported to be insect phagostimulants. During its life, an insect will need to ingest a particular amount and blend of different nutrients if it is to perform optimally. By use of olfactory, visual and tactile cues an insect is able to detect the plant with the highest quantities of the above nutrients developing a preference towards such a crop (Chapman and Bernays, 1995; Raffa *et al*., 2005).

The phagostimulatory power of the plant nutrition varies across and between plants affecting the insect pest preference (Cook, 2008; Dent, 2000).

Cassareto and Corcuera (1998) showed that, Dolichos species with the highest quantities of heat stable proteins had an effect on infestation of the plants by aphids and mites. The varieties with the highest quantities of these proteins were the most preferred while those with the least were unattractive to the phytophagous insects.

Members of Brassicaceae and other related families have been observed to vary in their insect herbivory properties, with some members being more resistant than others. This resistance is attributed to the biochemical and/or morphological characteristics of these family members. Spider plant has therefore shown variable host plant resistance to the

flea beetle which can be construed to be influenced by the biochemicals and/or morphological characteristics. (Moran and Hamilton, 1990; Wink, 2003)

In addition Simmonds *et al.*, (2002) was able to demonstrate that cruciferous crops with higher levels of carbohydrates, sugars, glucosinolates and phospholipids were most preferred by insect pests thus making them more susceptible. According to Mazza (2007), crops with low terpenoids were more susceptible to herbivory insects unlike those with high concentration of terpenoids.

The study was inspired by lack of specific information on the role played by metabolites in the damage inflicted by the flea beetles on the spider plant morphotypes. This is the case in most of the ALVs where despite their immense nutritional potential little research regarding their ecological, agronomical, metabolomical and genetic characteristics has been done. It was not until recently that these vegetables gained scientific attention as they were mostly referred to as weed plants. This neglect therefore led to introduction of exotic vegetables where much of the research was directed, this gradually resulted in these indigenous vegetables losing their ecological advantage becoming vulnerable to the introduced pests and diseases (Adango *et al.*, 2005; Abukutsa-Onyango, 2001; FAO, 1999). The study anticipated that just like the members of Brassicaceae family vary in their metabolites composition and the resulting pest damage, the Capparaceae family which the former may have evolved from after a long evolutionary process, may also vary in these aspects between its species as the latter has no scientific reference on host plant resistance qualities.

CHAPTER 3

MATERIALS AND METHODS

3.1 Seed collection and multiplication

Farmers from Rift Valley (Moiben and Kitale) and Western Province (Chavakali) provided the seed to be used in the experiment. The sites were chosen based on a previous survey conducted by Abukutsa-Onyango (2001). In the survey, valuable information on the scale of production, usefulness of the spider plant as a vegetable to the local people and reports by the local farmers that the flea beetles affected the foliage quality albeit in variable proportions among the morphotypes was obtained. Seeds of the three spider plant morphotypes were collected from the farmers' fields at the brown pod stage which is the harvest maturity stage and dried in the green house for two months to break the dormancy. The seeds were then sown in plastic pots (Ø 20 cm) inside the green house for seed multiplication. The seedlings were closely monitored throughout the growing period and similar morphotypes grouped together to obtain true to type morphotypes of purple, purple-green and green stemmed spider plant which were used for the experiment.

3.2 Experimental site and layout

A one year (May 2008-April 2009) host plant preference trial was set up in a screen house at the Department of Seed, Crop and Horticultural Sciences, University of Eldoret (0^0 30''N and latitude 35^0 15''E and 2140 MASL).

Experimental plastic pots containing planting media consisting of soil, farm yard manure and Di-Ammonium Phosphate (D.A.P) at a ratio of 60:30:0.5 kg respectively,

were placed on an area measuring 1 m x 2 m and a path of 50 cm left between the plots (Fig. 1). The experiment was laid in a CRD with three treatments, replicated six times. The treatments were the three spider plant morphotypes (purple, purple-green and green stemmed). Seed of the three spider plant morphotypes were planted in the pots and kept weed-free by uprooting any emerging weeds. The plants were thinned to 2 plants per pot at the 7^{th} week after planting (WAP).

3.3 Data Collection on Spider plant Damage

Flea beetles sourced from Dudu Technology Co. Ltd were introduced on the 7^{th} WAP at a rate of 5 beetles per plant. Before infestation, an insect screen was established in all replicates to prevent movement of beetles between the replications and also to shield the beetles from predators like the birds. Plate 3.

Data on foliage damage was taken after every 2 days for two weeks after infestation according to Chengwang, *et al*., (2001). The 2 days timing in data collection was selected based on the time required for the flea beetle to inflict significant damage, while the 7 weeks period taken before infestation of plant with flea beetles was determined by the time nutrients and biochemicals are at their optimum (Chweya, 1999).

While taking the data, number of 'shot holes' counted on all the leaves of the tagged plants where in each plot, 6 plants were randomly taken and tagged to be used in estimating the damage. Windows always resulted prior to holes emergence. But holes were used to express the actual damage; three leaves observed to be flea beetle damaged were selected and marked from the tagged plants and the average number of holes taken by physical counting through direct observation. For easy observation, a magnifying

glass was used to show the shot holes clearly. Bailey (1996) scoring scale was used for indexing the inflicted damage as shown in Table 3.

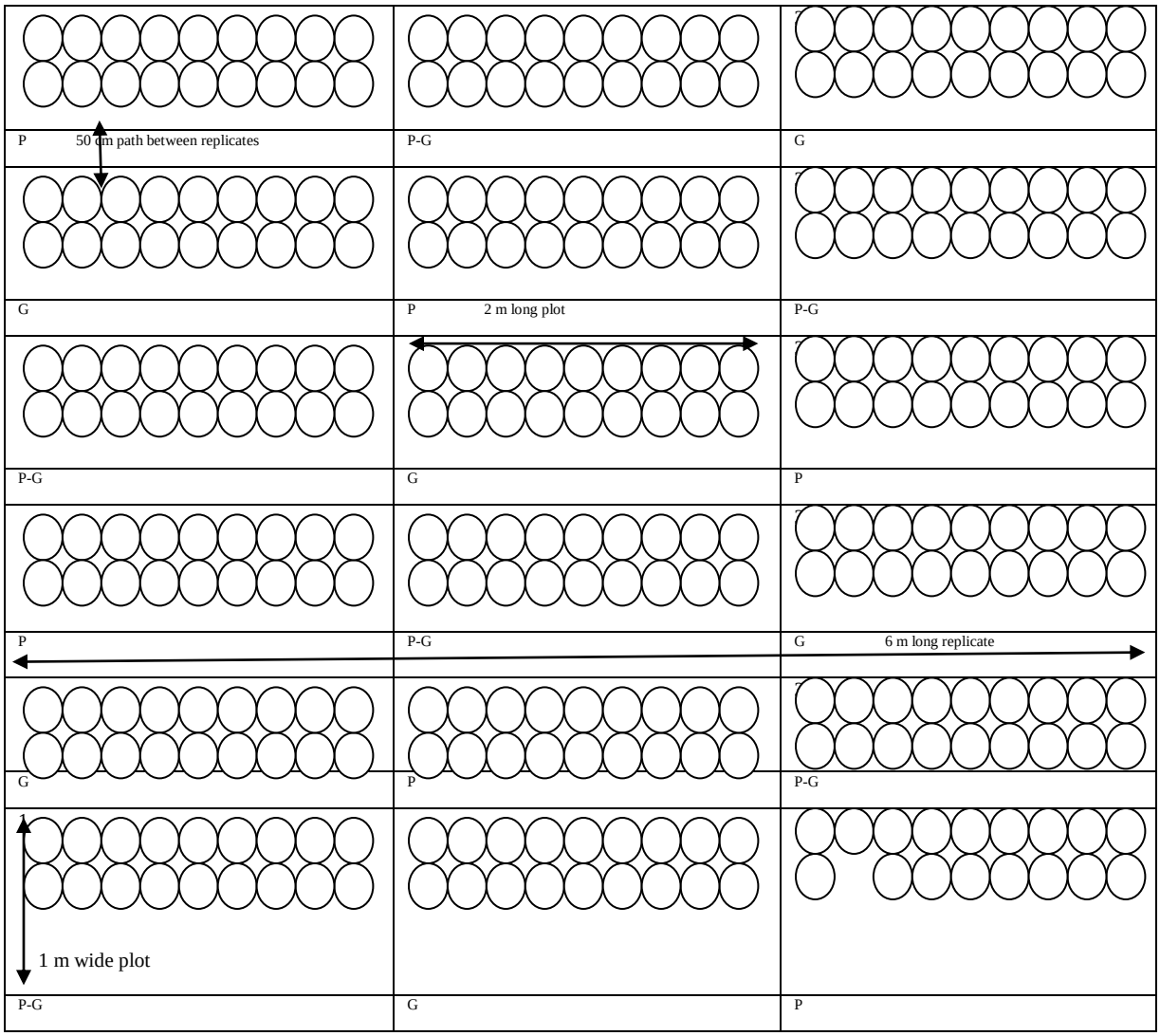


Fig.1 Field layout on infestation of the flea beetles on spider plant morphotypes.

KEY. P-Purple stemmed morphotype, P-G-Purple-green stemmed morphotype and G-Green stemmed morphotype.

(Source: Author, 2013)

Plate. 3 Experimental plots covered with insect screens.

(Source: Author, 2013)

Table 3. Bailey Scoring scale.

Scoring	Hole Scale	Index
1	0-3 holes/leaf	No- minimum damage
2	4-7 holes/leaf	Damage
3	>7 holes/leaf	High damage

(Source: Bailey, 1996)

3.4 Analysis of metabolites

The primary metabolites analysis was conducted in the Food Science Laboratory, while the secondary metabolites analysis was done in the Biochemistry Laboratory both in Jomo Kenyatta University of Agriculture and Technology (J.K.U.A.T)

Leaves of the three spider plant morphotypes were obtained from plants of each experimental plot after seven weeks of growth just before infestation with the flea beetles. The leaves from each morphotype and replicate were kept separately and dried to obtain the eighteen samples used for metabolite analysis.

3.4.1 Analysis of primary metabolites

The total primary metabolites were analysed using the proximate test for carbohydrates and micro-Kjedahl method for proteins as stipulated by Kirk, 2004 and Shida *et al.,* 1999. In the laboratory, the leaves were ground into fine powder using a miller machine.

Proximate analysis was carried out to obtain the quantities of carbohydrates, fat and proteins, where carbohydrate was obtained as difference of the moisture content, crude ash, crude fibre, and crude fat and crude protein.

3.4.1.1 Moisture Content

The steel containers were heated at 110^{o}C for 2 hours to obtain a constant weight. The container was cooled in a silica gel dessicator. A 20 g sample of ground spider plant morphotype was added to the container then placed to the oven, heated at 110^{o}C for 2 hours and placed in the dessicator to cool. The constant weight of the sample was taken and the moisture content calculated as a difference of the sample weight before drying and after drying in the oven. The same was repeated for all the samples and replicates.

3.4.1.2 Crude Fibre

A 20 g sample of ground spider plant morphotype was placed in a 500 ml beaker and dilute sulphuric acid added then placed in a refluxing chamber to digest the sample. The digest was filtered using a glass wool and rinsed with sodium hydroxide to remove the acid.

Refluxing was continued now using sodium hydroxide for 30 minutes the sample filtered and washed with boiling water. The residue was washed with dilute Hydrochloric acid and rinsed with water to remove the acid.

The residue was then washed once with absolute ethanol and three times with diethyl ether and dried in an oven at 100^{o}C for 1 hour then cooled in a dessicator.

The sample was then incinerated at 500^{o}C for 1 hour and cooled in a dessicator. The crude fibre was calculated as a difference of the original mass and the weight after desiccation for all the three samples. The procedure was repeated for all the samples and replicates.

3.4.1.3 Crude Ash

The crucible was heated at 550^{0}C for 1 hour and cooled in a dessicator then weighed. A 20 g sample of ground spider plant morphotype was placed in the dessicator for 2 hours; this was repeated until a constant weight was attained.

The crude ash was calculated as a difference of the weight before placing in the oven and the weight after heating in the oven. This was repeated for all the samples and replicates.

3.4.1.4 Crude Fat

A 20 g sample of ground spider plant morphotype was put in a thimble, placed in an oven at 100^{o}C for 1 hour and cooled in a dessicator. The sample was then added in to a round bottomed flask containing diethyl ether and heated at 110^{0}C for 18 hours, then cooled in a dessicator and weighed. Crude fat content was calculated as a difference of the sample weight before and after extraction of fat. The procedure was repeated for all the samples and replicates.

3.4.1.5 Crude Protein by micro-Kjedahl method

A 20 g sample of ground spider plant morphotype was placed in the digestion flasks, mixed with sulphuric acid and copper sulphate as the catalyst.

A blank test was prepared separately and sulphuric acid added. The two i.e. the sample and blank test were heated under a digestion heater until the colour changed to green like the blank. The samples were then transferred to distillation using the Marknan method and Titration done using hydrochloric acid until the orange colour occurred. The colour change was used to determine the amount of N per morphotype.

The amount of nitrogen was calculated which was then used to obtain the quantity of protein using the formula below.

N (%) = (VHCl*NHCl)-(VBK*NNaoH)-(VNaoH*NNaoH)\1.4007 W/100

Where;

VNaoH-mL standard NaoH needed to titrate the sample

VHCl-mLstandard HCl pipetted into titrating flask for sample

NNaoH-normality of NaoH

NHCl- normality of HCl

VBK-Ml standard NaoH needed to titrate 1 mL standard HCl minus B

B- mLstandard NaoH needed to titrate reagent blank carried through method and distilled into 1mL standard HCl

1.4007-milliequivalent weight of nitrogen *100

W-weight of the sample in grams.

F = Factor of Hcl, 0.99

N = Normality of Hcl, 0.02

S = Weight of samples

F-protein factor

(n) = percentage nitrogen (n)

Protein = (n)* (N)* 6.25* 100÷ (W)

3.4.1.6 Determination of carbohydrates

Carbohydrates content (C) calculation by difference

C= 100 – x

Where x = (weight in grams (Moisture content + crude ash + crude fibre + crude fat + crude protein in 100 g))

3.4.1.7 Determination of Beta – Carotene Content/Vitamin A

Standard solutions of beta carotene were prepared i.e. 1 ppm, 2 ppm, 4 ppm and 8 ppm and their absorbance on an ultra – violet – VIS spectrometer used in calibration.

A 20 g sample of ground spider plant morphotype was placed in 100 ml flask and heated with acetone and acidified sand for extraction.

The extract was concentrated with the rotary evaporator and eluted in petroleum ether. The elute was read under ultra-violet – VIS at 440 mm to obtain their respective absorbance values. A calibration curve was obtained as a linear equation from which the beta-carotene values in the samples were obtained. The procedure was repeated for all the samples and replicates.

$$1/Y(X) = 94.6925 * \text{and } (1/X)^2 + 53.56752 * (1/X) + 0.57010; R2 = 0.9938.$$

3.4.1.8 Determination of Ascorbic acid/ Vitamin C

Standardization of the indophenol solution was done and the ascorbic acid equivalent calculated.

A 20 g sample of ground spider plant morphotype was placed in a flask, acidified and Trichloroacetic acid (TCA) solution added. Then calculation of ascorbic acid equivalent filtration was done and 10 ml of each extracted sample titrated with indophenol solution. The amount of the filtrate used for the colour of the indicator to turn pink was taken and used to obtain the concentration of the ascorbic acid in the samples. The titration was repeated twice and the results agreed within 0.1 ml. The procedure was repeated for all the samples and replicates and concentration of ascorbic acid determined.

3.4.2. Analysis of secondary metabolites

3.4.2.1 Terpenoids

To determine the constitutive terpenoids quantity, a 20 g sample of the powdered foliage material was extracted with 10 ml of methanol in water bath at 80^0C for 10 minutes. The condensed filtrate was then used for chromatography. The concentration of constituent terpenes in the morphotypes was determined by high power liquid chromatography (H.P.L.C.) following the procedure of Raffa (1999); William and Kinghorn, (2005) The terpenes were separated using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. After 24 h, the extract was removed with a Pasteur pipette and filtered through glass wool. The sample was washed in 400 µl hexane to remove any remaining terpenes. The wash solvent was removed with a Pasteur pipette and filtered through glass wool. The volume of extract was brought to 2 ml by addition of hexane. Twenty micro-litres of 10% paracymene was added to each extract as an internal standard.

The colour and refractive values of these spots were recorded with visible light of the high powered liquid chromatography (HPLC) after spraying the plates with anaisaldeyde-

sulphuric acid reagent and heating at 60°C oven temperature for the first 10 min and increased 10°C/min for 10 min to 160°C. The carrier gas, helium, was maintained at 30 cm/s. The concentration of terpenes was determined by integrating peak areas using a densitometer. The peaks output were printed during observation. The procedure was repeated for all samples and replicates. (Mallikharjuna *et al.,* 2007; Horwitz *et al*., 1998)

3.4.2.2 Glucosinolates

Total glucosinolates were determined by the sulfate method of Mc Ghee *et al.,* (1996); William and Kinghorn (2005). A 20 g of the powdered foliage material was extracted with 70% ethanol on a rotary shaker at 180 thaws/min for 10 hrs. 70% lead acetate was added to the filtrate and centrifuged by adding 6.3% sodium carbonate at 100 r.p.m for 10 minutes. The retained supernatant was dried, redisolved in chloroform and used for chromatography. The glucosinolates were separated using Et OAC-methanol-water (80: 10:10) solvent mixture. The colour and refractive values of the spots were recorded by observing under ultra-violet (U.V 254 mm) from a densitometer, to obtain the peak intensity and level. The procedure was repeated for all the samples and replicates (Mallikharjuna *et al.,* 2007, Horwitz *et al*., 1998).

3.4.3 Data analysis.

The level of damage on the three morphotypes by the flea beetles was based on the feeding holes on the tagged plant leaves of each morphotype. The number of feeding holes observed were transformed to log10 (n+1) before analysis to standardize the variance, where n represented the average number of holes observed per morphotype.

The transformed data were then subjected to ANOVA (Analysis Of Variance) and Pearson's correlation coefficient (r) used to measure the strength of the association

between the damage inflicted by the flea beetles and the plant metabolites. The means were separated using the Fischer's L.S.D (Least significant Difference) test. Analysis was done using the statistical package Genstat 7.22, 2005 (Verzani, 2005).

CHAPTER 4

RESULTS

4.1 Flea beetles damage on the spider plant morphotypes

The foliage damage by Flea beetles (*P. mashonana)* was different among the purple stemmed, purple-green stemmed and green stemmed spider plant morphotypes. The flea beetles fed on the leaves resulting in characteristic shot-holes arrowed (Plate 4).

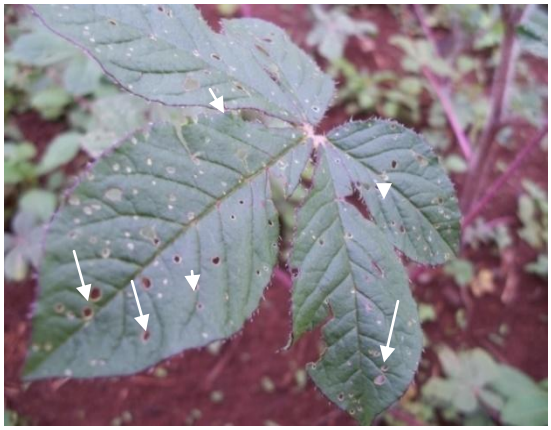


Plate. 4a Purple stemmed leaf-severely damaged.

(Source Author, 2013)

Plate. 4b Purple-green stemmed-moderately damaged.

(Source Author, 2013)

Plate. 4c Green stemmed morphotype-least damaged.

(Source Author, 2013)

The level of damage on the purple stemmed morphotype was significantly high. The green stemmed morphotype had the least damage. While the purple-green stemmed morphotype depicted a moderate damage level. The damage inflicted on the purple stemmed morphotype was three times severe than in the green stemmed morphotype. ($F \leq 26.76$, $P \leq 0.05$) (Fig. 2 and Appendix 1).

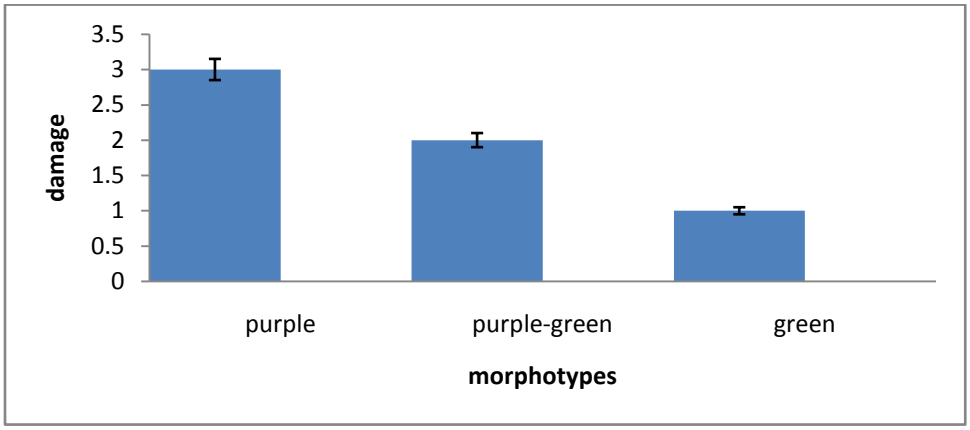


Fig. 2 Mean plant damage score by the flea beetles on the 3 spider plant morphotypes.

Where 1=minimum or no damage, 2=moderate damage and 3 =severe damage. All the differences in the damage are significant at $p \leq 0.05$ level. Error bars represent standard deviation.

In addition to the damage inflicted on the morphotypes being significantly different, there was a consistent variance of damage on all the sampling dates except the level of damage observed 2 days after infestation with the flea beetles. This was because of the time taken by the flea beetles to acclimatize and start feeding on the new host. The damage recorded

on the 14th day was more than tenfold the one observed two days after infestation of the flea beetles on the purple stemmed morphotype. However, the rate of damage decreased significantly after the 10th day with the recorded damage being insignificant as the flea beetles had reached their optimum feeding and feeding rate had reached the plateau (Table 4).

Table 4. Mean number of flea beetle damage holes on different spiderplant morphotypes for 14 days.

Treatment/Morphotype	Time (days)						
	2	4	6	8	10	12	14
Green stemmed	0.0 a	0.7 a	0.7 a	1.1 a	2.4 a	2.7b	2.7 b
Purple-green	0.0 a	0.9 a	2.5 b	4.4b	5.5 bc	7.2c	7.6c
Purple stemmed	0.5 a	1.6 ab	4.4 b	6.5 c	8.5 c	8.6c	8.6 c

Means linked with similar letters in a column are not significantly different $p \leq 0.05$.

4.2 Analysis of primary metabolites.

The foliage analysis of spider plant morphotypes revealed that the 3 morphotypes had different quantities of primary metabolites; carbohydrates, proteins and vitamins. It was observed that the purple stemmed morphotype had the highest concentration of carbohydrates (F=21.18, P=0.05), proteins (F=10.55, P=0.05) and Vitamins A and C (18.87 and 45.68, P=0.05 respectively). The purple-green stemmed morphotype had moderate concentration of these primary nutrients (carbohydrates, proteins and vitamins)

respectively. Lastly, the green stemmed morphotype had the least primary nutrients. The protein levels did not vary between the purple-green and green stemmed morphotypes but the carbohydrates levels showed the highest increase from the green to the purple stemmed morphotypes. (Table 5 and Appendices 2, 3, 5, 7 and 8).

Table.5 Percentages of primary nutrients in the spider plant morphotypes leaves (%).

Morphotype	Carbohydrates	Proteins	Fats	Vit.A	Vit.C
Green stemmed-1	1.7a	2.6a	1.4a	0.2a	14.3a
Purple-green -2	1.9a	2.8b	1.6b	0.29b	15.7b
Purple stemmed-3	2.5b	2.8b	1.7c	0.5c	16.5c

Means linked with different letters in a column are statistically different ($p \leq 0.05$).

4.3 Analysis of secondary metabolites

When the leaves of the spider plant morphotypes were analysed for the secondary metabolites, glucosinolates and terpenoids responsible for the damage by the flea beetle, it was found out that the purple stemmed morphotype had the highest concentration of glucosinolates 43% (Appendix 10). The purple-green stemmed morphotype had moderate glucosinolates quantities 37% (Appendix 11), while the green stemmed morphotype had the least glucosinolates 19% (Appendix 12) ($F=49.42$, $P=0.05$).

Conversely, the green stemmed morphotype had the highest terpenoids quantities 45% (Appendix 15), followed by the purple-green stemmed 35% (Appendix 14). The purple

stemmed morphotype had the least terpenoids quantities 15% (Appendix 13) (F=47.78, P=0.05). The variation of terpenoids in the morphotypes was higher than that of glucosinolates (Table 6 and Appendices 4, 6 and 10).

From the above analysis, it emerged that glucosinolates and terpenoids forms about 70% of the total secondary metabolites in the spider plant an indication that they play a major role in host plant preference.

Table 6. Percentages of secondary metabolites in the spider plant morphotypes foliage (%).

Morphotype	Glucosinolates	Terpenoids
Green stemmed-1	1.5a	2.0a
Purple-green -2	1.8b	2.2b
Purple stemmed-3	1.9c	2.9c

Means linked with different letters along a column are statistically different ($P \leq 0.05$).

4.4 Correlation of the flea beetle damage and plant metabolites

Results in Table 7 show that, primary and secondary metabolites in the spider plant morphotypes correlated with the flea beetle damage inflicted on the foliage.

A positive correlation was found between carbohydrates, fats, vitamins, proteins, glucosinolates and the inflicted damage (r=0.659*-1.000*) In contrast, terpenoids quantities correlated negatively with the inflicted damage by the flea beetles and the other metabolites.

There was a strong correlation between the metabolites and the damage inflicted by the flea beetle; this depicted the fact that, the damage inflicted on the spider plant morphotypes was more or less stimulated by the metabolites.

Table.7 Pearson's correlation coefficients of the relationship between spider plant morphotypes damage by the flea beetle and the metabolites.

	D	T	G	C	P	F	VC	VA
Damage (D)	1.000							
Terpenoids (T)	-0.794*	1.000*						
Glucosinolates(G)	0.805*	-0.951*	1.000*					
Carbohydrates (C)	0.754*	-0.685*	0.879*	1.000*				
Proteins (P)	0.659*	-0.745*	0.678*	0.786*	1.000*			
Fats (F)	0.760*	-0.945*	0.896*	0.645*	0.698*	1.000*		
Vit. C (VC)	0.830*	-0.894*	0.931*	0.688*	0.690*	0.688*	1.000*	
Vit. A (VA)	0.840*	-0.755*	0.780*	0.895*	0.715*	0.742*	0.759*	1.000*

All the r-values are significant (*) ($p<0.05$)

(-) Negative correlation.

CHAPTER 5

DISCUSSIONS

5.1 Flea beetle damage on spider plant morphotypes

The research clearly demonstrated that; there was significant difference in the level of flea beetle damage among the 3 morphotypes. The damage ranged from severe in the purple stemmed to minimal in the green stemmed, while the purple-green exhibited a moderate damage level. This study therefore, validates the Western Kenya and Rift Valley provinces communities' unreported observation that the flea beetle damage on the spider plant morphotypes is variable.

The variation of the damage in spider plant morphotypes is attributed to the differences in primary and secondary metabolites as found out extensively in this study. These metabolites play a major role in attracting or repelling the flea beetles which ultimately result in the damage (Traw, 2002).

5.2 Primary metabolites as phagostimulants.

Carbohydrates, proteins and vitamins act as major insect phagostimulants. This is informed by the fact that an insect will ingest a given amount of nutrients for optimum metabolism; the occurrence of these nutrients in high proportions in a particular plant species will result in a given insect pest preferring to feed on the plant to exploit the nutrient-ratio benefit (Arnod, 1996; Bartlet, 1996). This preferential feeding in turn results in damage, thus a plant containing high quantities of these nutrients will experience a high level of damage by the insect herbivores. This variation in primary metabolites (Carbohydrates, proteins and vitamins) can explain why the purple stemmed

morphotype with the highest concentration of the primary metabolites/nutrients was damaged most by the flea beetle *Phyllotreta mashonana*. The variation in damage can be attributed to the neurosensory ability of flea beetles to sense nutrients concentration in a host in order to derive maximum benefits from the high primary nutrients quantities. (Chapman and Bernays, 1995; Cook, 2008).

The nutritional sensory cues than any other sensory attribute (visual or touch) have been identified for host finding and acceptance. The molecular receptors for taste and smell are located on the peripheral sensory neurons of the antennae, mouth parts, tarsi and the ovipositor (Nielsen, 2005; Nielsen, 2011). Conversely, the low primary nutrient levels in the green stemmed morphotypes discouraged feeding as the flea beetles could not derive the same nutrient benefits as in the purple stemmed morphotype. This can be referred to as negative chemotaxis where a low concentration gradient exists between the morphotypes thereby repelling flea beetles from feeding on the morphotype thus least damage inflicted. The nutrient-benefit derived from the purple-green stemmed morphotype was moderate so was the feeding, attractiveness and damage on the morphotype by the flea beetles.

Another important attractant of flea beetles to the spider plant morphotypes, is the carbohydrate-protein ratio. The nutritional analysis of spider plant depicted that the purple stemmed morphotype had the highest carbohydrates-proteins ratio of 1.22:1.00. This attracted the flea beetles resulting in the highest damage level among the 3 spider plant morphotypes. The higher the ratio the more likely a given plant species will be preferred by the insect herbivores as found out by Barlet (1996). The purple-green stemmed morphotype had a carbohydrate-protein ratio of 1.20:1.00 which was a

moderate ratio while the green stemmed morphotype had the least carbohydrate-protein ratio of 1.15:1.00 and was therefore the least damaged. This nutrient-benefit feeding on spider plant morphotypes can be attributed to the fact that carbohydrates being the biggest source of energy for most organisms, an insect herbivore in this case the flea beetle will prefer to feed on the spider plant morphotype with the highest quantities of these nutrients. Similarly, proteins play a significant role in building the body of organisms, thus the spider plant morphotype with higher protein content will attract the flea beetles feeding in an attempt to gain most from ingestion of the morphotype. Vitamins also increase the immunity and suitability of an organism to the environment. In this regard, flea beetles will therefore aim at exploiting the host with the highest quantities of the vitamins in order to enhance their suitability to the environment. Thus, the primary metabolites offer an insect direct means of assessing the nutrient quality of a potential food which allows an insect using chemotaxis to feed more on the plant species considered more "nutritious" causing more damage (Kareiva, 1983; Barlet, 1996).

Supporting the above observation, Dixon and Payne (2006); Arnod (1996); Hiiesaar *et al.*, (2003) found out that primary metabolites play a significant role in phagostimulation, where insect herbivores will be attracted and feed more on the nutritious varieties than less nutritious ones. These nutrients therefore affect the insect herbivore gustatory responses thereby increasing the level of damage on the host plant. This therefore confirms the findings that there is a strong positive correlation between flea beetle damage and primary metabolites in spider plant morphotypes. High nutrients in the purple stemmed morphotype acted as a stimulant to the flea beetle feeding resulting in the highest damage on the foliage. The same primary metabolites significant reduction in the

green stemmed morphotype can be construed to have rendered the plant less desirable to the flea beetles thus the least foliage damage.

5.3 Effect of glucosinolates quantities in spider plant morphotypes on flea beetle damage.

Glucosinolates are water soluble group of secondary metabolites derived from glucose and amino acids and they contain sulphur and nitrogen in their structure (Van Dam *et al.,* 1998). These glucosinolates play an important role in host-pest interaction a condition that resulted after a long evolutionary process making glucosinolates a host specific attractant. Glucosinolates only affect specific insect herbivores either as attractants or repellents. Flea beetles feed only on plant containing glucosinolates a compound ubiquitous in the Brassiceae and the related families (Cosse *et al.,* 2006). This is because glucosinolates are considered host-specific stimulants derived from the dietary experience of the foraging pests (Renwick and Lopez, 2005). Glucosinolates play an identification role of the host to the flea beetles as they possess the necessary characteristic to trigger the physiological changes involved in development of dependence a phenomenon considered as a type of an "addiction" which is an adaptation of the flea beetles as they possess a nitrile-specifier protein (NSP) which attracts flea beetles to such plants. There is evidence that there exists a positive correlation between high quantities of glucosinolates and the resultant foraging of the plants by the flea beetles. This interaction is exceptionally complex with little knowledge on the genetic basis and the enzymes responsible for the interaction (Wittstock *et al.,* 2004).

Glucosinolates not only initiated but prolonged the feeding of the flea beetles which is attributed to the carbohydrate-protein rich background of glucose and amino acids which are readily absorbed forming an instant source of "succour" to the insect herbivore upon ingestion (Addesso, 2009). High quantities of glucosinolates in a given plant species will have a positive effect on the flea beetles infestation and damage (Hartmann, 2007). This explains why high quantities of these glucosinolates in the purple stemmed spider plant morphotype resulted in high feeding by the flea beetles inflicting higher damage than in the green stemmed morphotype which has low quantities of glucosinolates. The low glucosinolates in the green stemmed morphotype can therefore, be construed to have made the plant less desirable for feeding by the flea beetles thus the least damage inflicted on the leaves. Whereas, the purple-green stemmed morphotype was moderately damaged as the glucosinolates quantities were also found to be moderate.

A positive correlation was found between glucosinolates quantities and the damage inflicted by the flea beetles on the spider plant morphotypes. This confirms an earlier observation by Wittstock *et al.*, (2004) that high glucosinolates quantities results in an increased foraging by the flea beetles. This can be attributed to the fact that, glucosinolates play a major role in the desirability of the spider plant as host to the flea beetles in that, the flea beetle is host specific and the higher the quantities of these glucosinolates makes the host more suitable. This qualifies glucosinolates as strong phagostimulants. The correlation of glucosinolates and the flea beetle damage was very strong in this study (0.8) indicating that higher quantities of glucosinolates resulted in much feeding reflected as damage on the leaves.

However, experiments in controlled laboratory chambers by Bartlet, (1996), found out that a 5000 fold increase in glucosinolates concentration could only lead to a 7 fold increase in feeding by the flea beetles. This may be misleading as the experiment was done in a controlled environment, as the field conditions demonstrated that, provided there exists a significant difference in the concentration of glucosinolates in a particular host, then the flea beetle damage will be significantly different.

This damage may not be solely attributed to the glucosinolates, as most plants contain other phagostimulants which play a synergistic role in host plant selection in that, host plant desirability is a function of many factors. According to Hartmann (2007), flavonoids are readily ingested by herbivores and humans. These flavonoids are expressed as yellow pigments and plants with high contents of these flavonoids are preferred by many pests. Other phagostimulants include the carotenoids and anthocyanins.

5.4 Role of terpenoids quantity in spider plant morphotypes on flea beetle damage.

Terpenoids are aromatic secondary metabolites derived from 5-carbon isoprene units. These compounds are known for their insecticidal, antifungal, antibacterial and molluscicidal properties (Mallikharjuna *et al.,* 2007). Terpenoids form part of the volatile cues known as olfaction repellents of insect herbivores. Unlike the other metabolites i.e. primary metabolites and glucosinolates where taste allows evaluation of the nutritional potential prior to ingestion, terpenoids have a repulsive aroma to the flea beetles and when the flea beetles are attracted by other attractants, these terpenoids have a toxic effect on the flea beetles whereby upon ingestion, the compound inhibits A.T.P.

(Adenosine Tri-phosphate) formation, results in the alkalation of the nucleophiles, disruption of hormonal activity , complexation with the protein binding with free sterols, inhibition of respiration and/or increasing the relative electron partitioning to the alternative oxidizing pathway. When the terpenoids are ingested in large quantities, the above reactions may result in death of the insect herbivore (Gauthier *et al.*, 2009).

The characteristic of high terpenoids quantities makes the green stemmed morphotype the least attractive to the flea beetles which was reflected in form of the least damage inflicted on the leaves. This was in addition to the morphotype's less nutrient-benefit which made the flea beetles refrain from the morphotype as the "potential harm may not be worth the gain after consuming the host".

The repulsion of the flea beetles to the green stemmed morphotype was also informed by the well developed olfactory lobes at the antennae of the flea beetles which can sense high terpenoids levels in the morphotypes and "keep-off". The minimum damage inflicted on the green stemmed morphotype, can be attributed to the fact that, in the event of availability of a host plant albeit with toxic chemicals, some flea beetles may have developed resistance and may have a mechanism to sequester the toxic compounds and prolong living by ingesting the food, thereby justifying the observation that all the morphotypes had some feeding damage (Michael and Boyko, 2006).

The purple stemmed morphotype was the most suitable for the flea beetle feeding, owing to its low terpenoids quantities and high nutrients thus attracted flea beetles to feed on the foliage resulting in the highest damage inflicted.

A negative correlation was observed between the terpenoids, the flea beetle damage and the other metabolites. This can be explained by the fact that, when a host is said to be

desirable, most of the innate factors favour the pest. However, when a potential host is undesirable, most innate factors also deter the pests. In this study therefore, the green stemmed had the least desirable nutrients for attraction of flea beetles while at the same time had the highest feeding detterents in the form of terpenoids which resulted in the least damage inflicted on the morphotype.

According to Dicke and Baldwin, (2010), pest repellence in plants is also attributed to cyanogenic compounds like the cyanide which exists as an unstable compound and easily disintegrate releasing the highly toxic and volatile cyanide. This gas easily binds the nervous system of insect herbivores and other animals killing them instantly when the cyanide is in high concentration. As a result of this, many pests tend to avoid such plants unless endowed with a natural ability for countering the toxic cyanogenic compounds. Other invertebrate anti-feedant secondary metabolites include; atropines, phytic acid and gossypol all which have a negative effect on foraging pests.

Vincent and Stewart (1985), attributed host plant desirability to the foliage colour, in that, insects have inhererent colour preferences mostly those colours which resemble the foliage, flower or even hosts. Different insects are attracted by different host colours with most Lepidopterous preferring yellow colours of 50-560nm. Hymenoptera have colour receptor in blue and ultra-violet with some species also being able to perceive red (Lobdell *et al.,* 2005). The Coleoptera insects have a high recognition for pink, purple, ultra-violet and black colours (Anderbrant *et al.,* 2009). Members of the same family may prefer more than one colour like in the case of Curculionidae. In addition, colour preference may also differ at species and metamorphic level with some instars preferring

different colours at different stages (Simpson and Raubenheimer, 1995; Chen *et al.*, 2002; Addesso, 2009). The fact that flea beetle is a member of Coleoptera family may explain why the purple stemmed morphotype attracted the flea beetles to feed on its foliage resulting in higher damage than the green stemmed morphotype which was least attractive therefore had the least damage inflicted on the foliage. However, foliage colour cannot be the only host selection basis as insect herbivore damage is dependent on plant nutrient-benefit and chemotaxis.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

i. The purple stemmed morphotype was the most severely damaged while the green stemmed morphotype had the least damage. The purple-green stemmed morphotype was moderately damaged.

ii. The contents of plant metabolites also varied across the morphotypes, the purple stemmed morphotype had the highest primary metabolites (carbohydrates, proteins and vitamins) followed by the purple-green stemmed morphotype, the green stemmed morphotype had the least primary metabolites.

iii. In addition to the primary metabolites, the morphotypes had varying quantities of secondary metabolites with the purple having the highest glucosinolates and the least terpenoids. The purple green stemmed had moderate quantities of glucosinolates and terpenoids while the green stemmed had the least quantities of glucosinolates and the highest quantity of terpenoids.

iv. Flea beetle damage in spider plant increased with the increasing primary metabolites and glucosinolates while it decreased with increasing terpenoid levels.

6.2 Recommendations

i. The green stemmed morphotype can be crossed with the more nutritious but flea beetle susceptible purple stemmed morphotype in order to increase yield and maintain the desirable nutritional potential of the morphotype.

ii. Farmers can always grow the green stemmed morphotype in areas with high incidences of flea beetles as it is less damaged, also the purple stemmed can be alternately grown with the green stemmed morphotype to reduce build up of the flea beetles in the farm.

REFERENCES

Abukutsa-Onyango, M.O. (2001). Market survey on African indigenous vegetable in Western Kenya. In: Proceedings of the 2nd Horticulture Seminar on Sustainable Horticultural Production in the Tropics**,** J.K.U.A.T.

Abukutsa-Onyango, M.O. **(**2003). Unexploited Potential of Indigenous African Vegetables in Western Kenya. *Maseno Journal of Education, Arts and Science* 4: 103-122.

Abukutsa-Onyango, M.O. (2004). The Diversity of Cultivated African Leafy Vegetables in Three Communities in Western Kenya. *Maseno Journal of Education, Arts and Science* 7 (3): 229-231.

Abukutsa-Onyango, M.O. (2009). Solving Kenya's Food Crisis, One Indigenous Crop at a Time. *African Journal of Food Agriculture, Nutrition and Development* 7: 78-99.

Adango, E., Onzo, A., Hanna, R., Atachi, P. and James, B. (2005). Pests of Major Importance on Indigenous Leafy Vegetables. The Search for Appropriate Control Strategies. International Conference on Indigenous Vegetables and Legumes. Horticulture 752: 311-328.

Addesso,K. (2009). Pepper Weevil Attraction to Volatiles from Host and Non-host Plants. *Environmental Entomology* 38: 216-224.

Agerbirk, N., Olsen, C. and Nielsen, J. (2001). Season Variation in Leaf Glucosinolates and Insect Resistance in two types of *Barbarea vulgaris*. *Phytochemistry* 58: 91-100.

Agerbirk, N., Orgaard M. and Nielsen, J. (2003). Glucosinolates, Flea Beetle Resistance, and Leaf Pubescence as Taxonomic Characters in Genus *Barbarea (Brassicaceae) Phytochemistry* 63: 69-80.

Agerbirk, N. (2011). Responses of The Flea Beetles *Phyllotreta nemorum* and *P.cruciferae* to metabolically engineered *Arabidopsis thaliana* with an altered glucosinolate profile. *Chemoecology* 11: 75-83.

Anderbrant, O., Lolquist, J., Jonssen, J. and Marling, E. (2009). Effects of the Pheromone Trap Type, Position and Colour on the Catch of the Pine Sawfly. *Journal of Applied Entomology* 107: 1-5.

Arnod, R. 1996. Feeding and Oviposition Preferences of White Pine Weevil (Coleoptera: Curculionidae) on Resistant and Susceptible Sitka Spruce Clones in Laboratory Bioassays. *Environmental Entomology* 25: 1012–1019.

AVRDC. (2000). Asian Vegetable Research and Development Centre Publication and Fact Sheets and Indigenous Vegetables Spider Plant.

Bartlet, E. **(**1996). Chemical Cues to Host-plant Selection by Insects of Oil Seed Rape. *Agricultural Zoology Reviews* 7: 89-96.

Bartlet, E. Parsons, D. Williams, I. and Clark, S. (2011). The Influence of Glucosinolates and Sugars on Feeding by The Cabbage Stem Flea Beetle, *Psylliodes chrysocephala*. *Entomologia Experimentalis et Applicata* 73: 77-83.

Bailey, L. (1996). Effects of Simulated and Flea Beetle Injury to Cotyledons on Growth of Drought-stressed Oils Seed Rape, *Brassica napus L*. *Canadian Journal of Plant Sciences*. 77: 475–481.

Beran, F. (2011). Host Preference and Aggregation Behavior of The Striped Flea Beetle, *Phyllotreta striolata*. Diploma Thesis, Humboldt-University, Berlin. 30: 41-47.

Boulter, N. **(**2008). Assessing The Susceptibility of Crucifer Seedlings to Flea Beetle *(Phyllotreta spp.)* Damage. *Canadian Journal of Plant Sciences* 68: 85–93.

Bycznski, L. (1999). Tips, but no easy answers to Flea Beetle Invasions. Journal of Agriculture, Science and Technology: Volume 8 pp.12.

Cassareto, J. and Corcuera, L. (1998). Proteins Inhibits Accumulation in Aphid Infested Barley Leaves. *Phytochemistry* 80: 2279-2285.

Chapman, R. and. Bernays, E. **(**1995). *Insect* Behavior at The Leaf Surface and Learning as Aspect of Host Selection. *Journal of Experimental Biology* 45: 215-222.

Chen, Z., Kolb, T., and Clancy, K. (2002). The role of Monoterpenes in The Resistance of Douglas fir to Western Spruce Budworm Defoliation. *Journal of Chemical Ecology*. 28(5): 897-920.

Chengwang, P., Michael, W. and Mark, A. **(**1998). Flea Beetle (Coleoptera: Chrysomelidae) Response, Feeding, and Longevity on Oil Seed Rape and Crambe. *Environmental Entomology*. 21: 604-609.

Chigwumira, N., Mvere, B and Mhanzo, M. (1998). Traditional Vegetable Improvement Project Agronomic Traits. *African Journal of Food Agriculture, Nutrition and Development*.7: 613-620.

Chweya, J. (1997). Genetic enhancement of indigenous vegetables in Kenya. In :Guarino,L (ed.). Traditional African Vegetables. Proceedings of the IPGRI International workshop on Genetic Resources of Traditional Vegetables in Africa : Conservation and Use. Nairobi, Kenya. IPGRI, Rome. pp 86-95.

Chweya, J. (1999). Genetic enhancement of indigenous vegetables in Kenya. Paper presented at workshop "Genetic Resources of Traditional Vegetables in Africa: Options for Conservation and Use. Nairobi, Kenya 20: 120-128.

Chittenden, F. and Marsh, H. (1990). The Western Cabbage Flea Beetle. USDA, Washington D.C. Bulletin No. 902. pp 21.

Cook, K. (2003). Seasonal Fluctuations in Population Densities of The Corn Flea Beetle, *Chaetocnema pulicaria* (Coleoptera: Chrysomelidae) And their Impact on The Incidence of Stewarts wilt in Sweet Corn. M.S. thesis, University of Illinois. Urbana, IL. pp 27-57.

Cook, A. **(**2008**).** Nutrient chemicals as phagostimulants for *Locusta migratoria.* L. *Ecological Entomology* 2: 113-121.

Cosse, A., Bartelt, R., Zilkowski, B., Bean, D. and Andress, E. (2006). Behaviorally active green leaf volatiles for monitoring the leaf beetle *Diorhabda elongata*, a biocontrol agent of salt cedar. Journal of Chemical Ecology. 32: 2695- 2708.

De Jong, P., Hanne, O., Frandsen, H., Rasmussen, L., and Nielsen, J. (2000). Genetics of resistance against defences of the host plant Barbarea vulgaris in a Danish flea beetle population. *Proc Biol Sci*. 267 (1453): 1663-1670.

Dent, D. **(**2000). Integrated Pest Management. (2nd edition) CABI. Publishing Wellingford pp 425.

Dicke, M. and Baldwin, I. (2010). The evolutionary context for herbivore induced plant volatiles: beyond the "cry for help". *Trends in Plant Science* 15 (3): 167-175.

Dixon, W. and Payne, T. (1980). Attraction of entomophagous and associate insect of the southern pine beetle to beetle and host- three produced. *Journal of Entomologia Experimentalis et Applicata* 15: 378-389.

FAO. (1999). Utilization of tropical foods, fruits and vegetables. Food and Vegetable Nutrition paper 47/7. Rome, Italy.

Feeny, P., Standler, E.,Ahman, I. and Carter, M. (1989). Effects of plant odor on oviposition by the black shallow tail butterfly, *Papilio polyxenes*. *Insect Behaviour*. 2: 803-827.

Finch, S. (1980). Chemical attraction of plant-feeding insects to plants. Application Biology. 5: 67-143.

Fische, N.H. (1991). Plant terpenoids as allelopathic agents. *Phtytochemistry* 10: 659-678.

Gauthier, C., Legault J.,Girard-Lalancette K., Mshvildadze V, and Pichette A. (2009). Haemolytic activity, cytotoxicity and membrane cell permeabilisation of synthetic and natural and lupine-and oleanane-type saponins. *Medical Chemistry* 17: 2002-2008.

Giamoustaris, A, and Mithen, R. (1995). Modifying the leaf glucosinolate content of oilseed rape and its effect on its interaction with specialist and generalist pests. *Annals of Applied Biology*. 126: 347–363.

Hall, R. **(**2006). Plant metabolomics:from holistic hope,to hype,to hot topic. *New Phytochemistry*. 169: 453-468.

Hartmann, T. (2007). "From waste products to Ecochemicals: Fifty years research of plant secondary metabolism" *Phytochemistry*. 68. 22-24.

Hiiesaar K., Metspalu L. and Jogar K. (2006). Attractiveness and susceptibility of *Brassica rapa, B. napus and Sinapis albato* to the flea beetles (Coleoptera: Chrysomelidae). *Agronomy Research*. 4: 191–196.

Horwitz, W., Briton, P. and Chirtel, S. (1998). A simple method for evaluating data from and interlabolatory study. Journal of AOAC International. 81 (6) 1257-1265.

Imbamba, S.K., Ndawula, M.S. and Papa, G. (1977). The effect of soil moisture stress on photosynthesis, transpiration and leaf enlargement in some Kenyan vegetable plants. *East Africa Agriculture and Forestry Journal* 42 (3): 309-315.

Jansen, J., Allwood J., Marsden-Edwards E., Van der Putten W., Goodacre R. and Van Dam N. **(**2009). Metabolomic analysis of the interaction between plants and herbivores. *Metabolomics* 5: 150-161.

Kareiva, P. (1983). Influence of vegetation texture on herbivore populations: resource concentration and herbivore movement, pp. 259-289. *In* R. Denno (ed.) Variable plants and herbivores in natural and managed systems. New York: Academic Press.

KFSSG (Kenya Food Security Steering Group (2009) Report.

KENRIK (Kenya Resource Centre for Indigenous Knowledge) Report. (2004), National Museums of Kenya.

Kimiywe, J., Waundo, J., Mbithe, D., and Maundu, P. (2007). Utilization and medicinal Value of indigenous leafy vegetables consumed in Urban and Peri-Urban Nairobi. *African Journal of Food Agriculture Nutrition and Development*. pp 7.

Kirk, H. **(**2004). Compairing Metabolomes the chemical consequences of hybridization in Plants. *New Phytochemistry* 167: 613-622.

K'Opondo, F.B.O. (2009). Characterisation and seed quality aspects of selected types of Spider plant from Western Kenya. PhD Thesis. Department of Seed, Crop and Horticultural Sciences, Moi University. pp 5-12.

K'Opondo, F.B.O. (2011). Morphological characterisation of selected spider plant types From Western Kenya. *Annals of Biological Research* 2: 54-64.

Kuzina, V., Ekstrøm, T., Andersen, S., Nielsen, J., Olsen, C. and Bak, S. (2009) Identification of Defense Compounds in *Barbarea vulgaris* against the Herbivore *Phyllotreta nemorum* by an Ecometabolomic Approach. *Plant Physiology*. 151: 1977-1990.

Lobdell, C., Yong Tze-Hei and Hoffmann, M. (2005). Host colour preferences and Short-range searching behaviour of the egg parasitoid *Trichogramma ostriniae*. *Entomologia Experimenta et Applicata*. 116:127-134.

Louda S. and Mole, S. (1991). Glucosinolates: Chemistry and Ecology. In. Rosenthal, G. and Berebaum, M. (eds) Herbivores their interactions with secondary plant metabolites 1. The Chemical Participants: Academic Press, London. pp 123-164.

Mallikharjuna, P.; Rajanna, L.; Seetharam, Y. and Sharanabasappa, G. (2007). Phytochemical studies of *Strychnos potatorum* L. medicinal plant. *E-Journal of Chemistry* (4): 510-518.

Maundu, P., Achigan-Dako, E. and Morimoto, Y. (1996). Promoting the conservation and use of underutilized and neglected crops. Proceedings of the International Plant Genetic Resources Institute workshop.

Maundu, P. **(**1997). The status of traditional vegetable utilisation in Kenya. In :L Guarino (Ed). Proceedings of the IPGRI International workshop on genetic resources of Traditional vegetables in Africa: Conservation and use. ICRAF- HQ, Nairobi, Kenya: pp 66-75.

Mazza, G. (2007). Saponins: properties, applications and processing. *Critical Review Food Sciences and Nutrition* 47:231-258.

Mbugua, G., Gitonga, L.N., Gatambia, E., Mureithi J., Karoga, J. and Manyeki, L. (2005). Farmer participatory prioritization and development of agronomic practices of African leafy vegetables in Thika and Machakos districts. Paper presented in the 5th workshop on sustainable Horticultural production in the tropics. Egerton University, Njoro-Kenya.

Mc Caffrey J. (2004). Assessment of *Sinapis alba*, *Brassica napus* and *S. alba* x *B. napus* Hybrids (Coleoptera: Curculionidae). *Journal of Agricultural Science* 132:289-295.

Mc Ghee, D., Munkvold, G., and Iles A. (1996). Effects of imadacloprid seed treatment of corn on foliar feeding and *Erwinia stewartii* transmission by the corn flea beetle. *Plant Disease*. 80: 747-749.

Michael, S. and Boyko, E. (2006). The molecular bases of plant resistance and defense responses to aphid feeding: current status Department of Entomology, Kansas State University, Manhattan, KS 66506-4004.

Mitchell, B. **(**1999). The chemosensory basis of host plant recognition in Chrysomelidae. In P.H. Jolivet, M.L. Cox & E. Peptitpierre (eds.) Novel aspect of the Biology of Chrysomelidae. Kluwer Ademic Publishers, Dordrecht: pp141-151.

Moran, N. and Hamilton, W. (1990). Low nutritive quality as defense against herbivores. *Journal of Theoretical Biology* 80: 247-254.

Mnzava, N. (1999). Studies on tropical vegetables and amino acids composition in seed of *Cleome gynandra*.L. Selections from Zambia. *Food Chemistry*. 35(4): 287-293.

Mnzava, N. and Chigumira F. (2004). *Cleome gynandra* L In: Grubben, G.J.H. & Denton, O.A. (Eds). Plant Resources of Tropical Africa 2. Vegetables. PROTA Foundation, Wageningen, Netherlands/ Backhuys publishers, Leiden, Netherlands/CTA, Wageningen, Netherlands: pp 191-195.

Nekesa, P. and Meso, B. (2005).Traditional African vegetable production, marketing and utilization in western Kenya. Paper presented at the workshop "Genetic Resources of Traditional vegetables in Africa. Options for Conservation and Use. pp 29-31.

Nielsen J. K., Hansen M. L., Agerbirk N., Petersen B. L. and Halkier B. A. (2001). Responses of the flea beetles *Phyllotreta nemorumand*, *P*. *cruciferae* to metabolically engineered *Arabidopsis thaliana* with an altered glucosinolate profile. *Chemoecology*. 11 (2): 75–83.

Nielsen, J. (2005). Crucifer-feeding Chrysomelidae: Mechanism of host plant finding and acceptance, in: P. Jolivet, E. Petitpierre, & T.H. Hsiao (eds), Biology of Chrysomelidae. Kluwer Ademic Publishers, Amsterdam. pp 25-40.

Nielsen, J. (2011). Host plant discrimination within Cruciferae. Feeding responses of four leaf beetles (Coleoptera. Chrysomelidae) to glucosinoaltes, cucurbitacins and cardenolides. *Entomologia Experimentalis et Applicata*. 24: 41-54.

Omondi, C. **(**1989). Variation and yield prediction analyses of some morphological traits in six Kenyan landraces population of spider plant (L.) Briq). MSc Thesis, University of Nairobi, Nairobi, Kenya.

Onyango, M.A. (2001). Unexploited potential of indigenous African vegetables in Western Kenya. Maseno University Conference Proccedings. pp 41.

Opole, M, Chweya, J. and Imongi, J. (1995). Indigernous vegetables of Kenya, indigenous. Knowledge, agronomy and nutritive value. Field and Laboratory Experiments report. pp. 313-314.

Peterson, C., Cosse, A. and Coats, J. (2000). Insecticidal components in the meal of *Crambe abyssinica*. *Journal of Agriculture and Urban Entomology* 17: 27-36.

Pilson, J. (2007). The complexity of factors driving volatile organic compounds. Biological Plants. 66, 24-40.

Raffa, K. (1999). In vitro analysis of MT-based vesicle transport by centrifugal reflection microscopy. *Journal of Phytochemistry*. 96: 52-83.

Raffa, K., Krause, S., and Reich, P. (2005). Long-term effects of defoliation on red Pine suitability to insects feeding on diverse plant tissues, *Chemoecology* 79 (7): 2352-2364.

Rao, A. and Rajendrudu, G. (1999). Net photosynthetic rate in relation to leaf anatomical characteristics of C-3, C-3-C-4 and C-4 dicotyledon. Proceedings of Indian Academic Sciences. *Plant Sciences*. 99 (6): 529-538. 00.

Raubenheimer, D. and Simpson, S. (1993). Rails and arcs: The geometry of feeding. *Animal Behavior* 45: 953-964.

Renwick, J. (2002). Ovipositional bahaviour in Lepidoptera. *Annals Review of Entomology*. 39: 377-400.

Renwick, J. and Lopez, K. (2005). Experience based food consumption by larvae of *Pieris rapae*: Addiction to glucosinolates. *Entomologia experimentalis et Applicata.* 91: 359-68.

Rodman, J., Price, R., Karol, K., Conti, E., Sytsma, K. and Palmer, D. (1993). Nucleotide sequences of the rbcL gene indicate monophyly of mustard oil plants. Annals of. Missouri Botanical Garden. 80: 686-699.

Schippers, P. (2002). African indigenous vegetables: An overview of the cultivated species, Natural Resources Institute/ACP-EU Technical Centre for Agricultural and Rural Cooperation, Chatham, UK. pp 51-59.

Simmonds, M., Simpson, S. and Blaney, T. (2002). Dietary selection behaviour in *Spodoptera littoralis. Biochemistry* 3: 23-41.

Simpson, S. and Raubenheimer R. **(**1995). Feeding behavior, sensory physiology and nutrient feedback. *Experimental Biology* 13: 55-60.

Shida, H., Suzuno; H., Sugyama, N., Innami, S., Idadokoro, I. and Aekawa, A. (1999). Nutritive evaluation on chemical components of leaves stalks and stems of sweet potatoes (*Ipomea batatas*) *Plant Biochemistry* 38: 133.

Shinoda, T., Nagao T., Serizawa H. and Kawai, A. (2003). Identification of terpenoid saponins from a Crucifer, *Babarea vulgaris*, as a feeding deterrent to the diamond black moth, *Plutella xylostella. Journal of Chemical Ecology* 28: 587-599.

Spencter, J. (2009). Synergism in ovipositional behaviour of insect herbivores. *Journal of Insect Behavior*. 12: 483-500.

Sparg, S., Light, M., Van Staden, J. (2004). Biological activities and distribution of plant sapoins. *Journal of Ethnopharmacology*. 94: 219-243.

Tentelier, T. (2007). Jasmonate deficient plants have reduced direct and indirect defenses against herbivores. *Ecology Letter*. 764-778.

Thompson, L. **(**2005). Headspace volatiles of whole plants and macerated plants parts of *Brassica* and *Sinapis*. *Phytochemistry*. 27: 4013-4018.

Traw, M. (2002). Constitutive and induced resistance to insect herbivores of *Brassica nigra*: a test of optimal defense theory. Dissertation, Cornell University, I thaca, New York, USA. pp 38-51.

Van Dam, N., Hans E., Van Dam. K., Gert C., Van H., Gerrit J., Raaijmakers R., Natasja C., Postma E., Groeneweg B., and Jongen W. (1998). "The glucosinolates are important determinants for taste preference and bitterness of Brussels sprouts". *Journal of the Science of Food and Agriculture* **78**: 30.

Verma, S. and Pandey, H. (1998). Biochemical and biological variation of southern pine Beetle populations in North and Central America. *Journal of Agricultural. Entomology*. 75: 422–435.

Verzani, J. (2005).Using R for introductory statistics, 3rd edn. Chapman and Hall/CRC Press. pp 3455-3478.

Vincent, C. and Stewart, R. (1985) Influence of trap colour on capture of adult crucifer feeding flea beetles. *Journal of Agricultural Entomology*. 33: 320-327.

Waswa, P; Obiero, H., Otukho, S and Eclangwa, M. (1996). Response of various local vegetables to different types/rates of manure. *Africa Journal of Agriculture*. 62: 120-140.

Wety, K. (2007). Influence of plant allelochemicals on the diamond back moth in Brassicas. *Ecology*. 61: 1478-1500.

William, P. and Kinghorn, A. (2005). Extraction of secondary metabolites. *Biotechnology*. 20: 323-351

Wink, M. (2003). Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry* 64: 3–19.

Wittstock, U., Agerbirk N., Stauber E.J., Olsen C.E.,and Hippler, M. (2004). Successful herbivore attack due to metabolic diversion of a plant chemical defense. Proceedings of Natural Academic Sciences, USA. 101: 4859–64.

APPENDICES

Appendix 1. Analysis of variance for beetle-damage in spider plant morphotypes.

Source	d.f.	s.s.	m.s.	F	Sig.
var	2	10.1111	5.0556	26.76	0.05
Residual	15	2.8333	0.1889		
Total	17	12.9444			

Grand mean 1.94 s.e.d. 0.251 l.s.d (0.05) 0.535 c.v 22.4%

Identifier	Mean
Green (1)	1.000
Purple-green (2)	2.000
Purple (3)	2.833

Appendix.2 Analysis of variance for carbohydrates in spider plant moprphotypes.

Source	d.f.	s.s.	m.s.	F	Sig.
var	2	2.10010	1.05005	21.18	0.05
Residual	15	0.74355	0.04957		
Total	17	2.84365			

Grand mean 2.072 s.e.d. 0.1285 l.s.d (0.05) CV 10.7%

Identifier	Mean
1	1.735
2	1.940
3	2.540

Apendix.3 Analysis of variance for fats in the spider plant morphotypes.

Source	d.f.	s.s.	m.s.	F	Sig.
var	2	0.142811	0.071406	63.95	0.05
Residual	15	0.016750	0.001117		
Total	17	0.159561			

Grand mean s.ed l.s.d 0.05 CV% 2.1

Identifier	Mean
1	1.462
2	1.613
3	1.673

Appendix.4 Analysis of variance for glucosinolates in the spider plant morphotypes.

Source	d.f.	s.s.	m.s.	F	Sig.
var	2	0.502533	0.251267	49.42	0.05
Residual	15	0.076267	0.005084		
Total	17	0.578800			

Grand mean 1.723 s.e.d. 0.0412 l.s.d (0.05) CV 4.1%

Identifier	Mean
1	1.507
2	1.750
3	1.913

Appendix.5 Analysis of variance for proteins in the spider plant moprphotypes.

Source	d.f.	s.s.	m.s.	F	Sig.
var	2	0.087544	0.043772	10.55	0.05
Residual	15	0.062233	0.004149		
Total	17	0.149778			

Grand mean 2.72 s.e.d. 0.0372 l.s.d (0.05) 0.0793 CV 2.4%

Identifier	Mean
1	2.625
2	2.750
3	2.788

Appendix.6 Analysis of variance for terpenoids in the spider plant morphoytypes.

Source	d.f.	s.s.	m.s.	F	Sig.
var	2	2.823644	1.411822	47.78	0.05
Residual	15	0.044317	0.002954		
Total	17	2.867961			

Grand mean 2.367 s.e.d. 0.0314 l.s.d (0.05) 0.0669 CV 2.3%

Identifier	Mean
1	2.005
2	2.178
3	2.918

Appendix.7 Analysis of variance for Vit_A in spider plant morphotypes.

Source	d.f.	s.s.	m.s.	F	Sig.
var	2	0.3208111	0.1604056	18.87	0.05
Residual	15	0.0127500	0.0008500		
Total	17	0.3335611			

Grand mean 0.3572 s.e.d. 0.01683 l.s.d (0.05). 0.03588 CV 8.2%

Identifier	Mean
1	0.2367
2	0.2917
3	0.5433

Appendix.8 Analysis of variance for Vit_C in spider plant morphotypes.

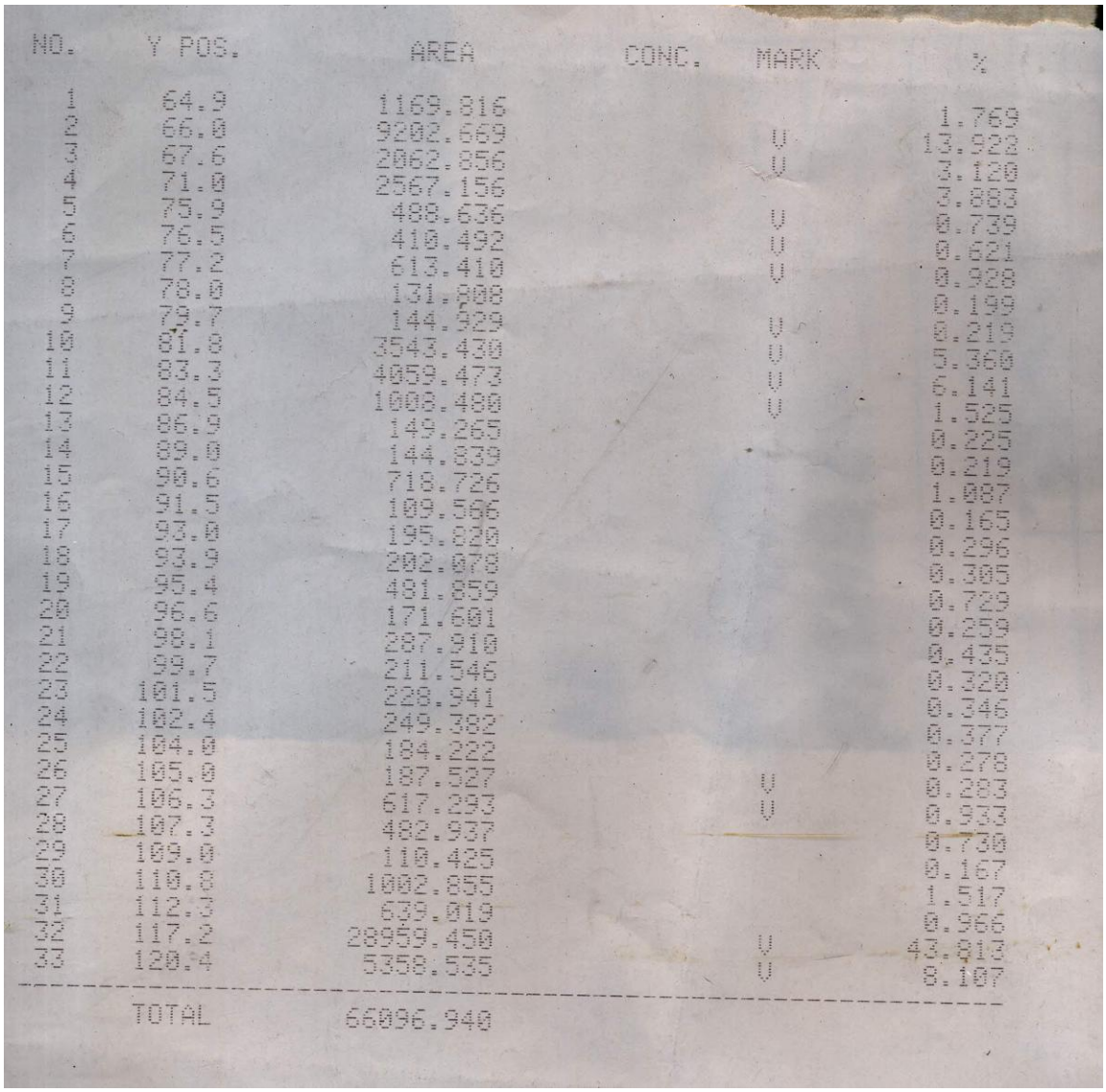
Source	d.f.	s.s.	m.s.	F	Sig.
var	2	15.9992	7.9996	45.68	0.05
Residual	15	2.6267	0.1751		
Total	17	18.6259			

Grand mean 15.481 s.e.d. 0.2416 l.s.d (0.05) 0.5150 C.V 2.7%

Identifier	Mean
1	14.25
2	15.66
3	16.54

Secondary metabolites HPLC output.

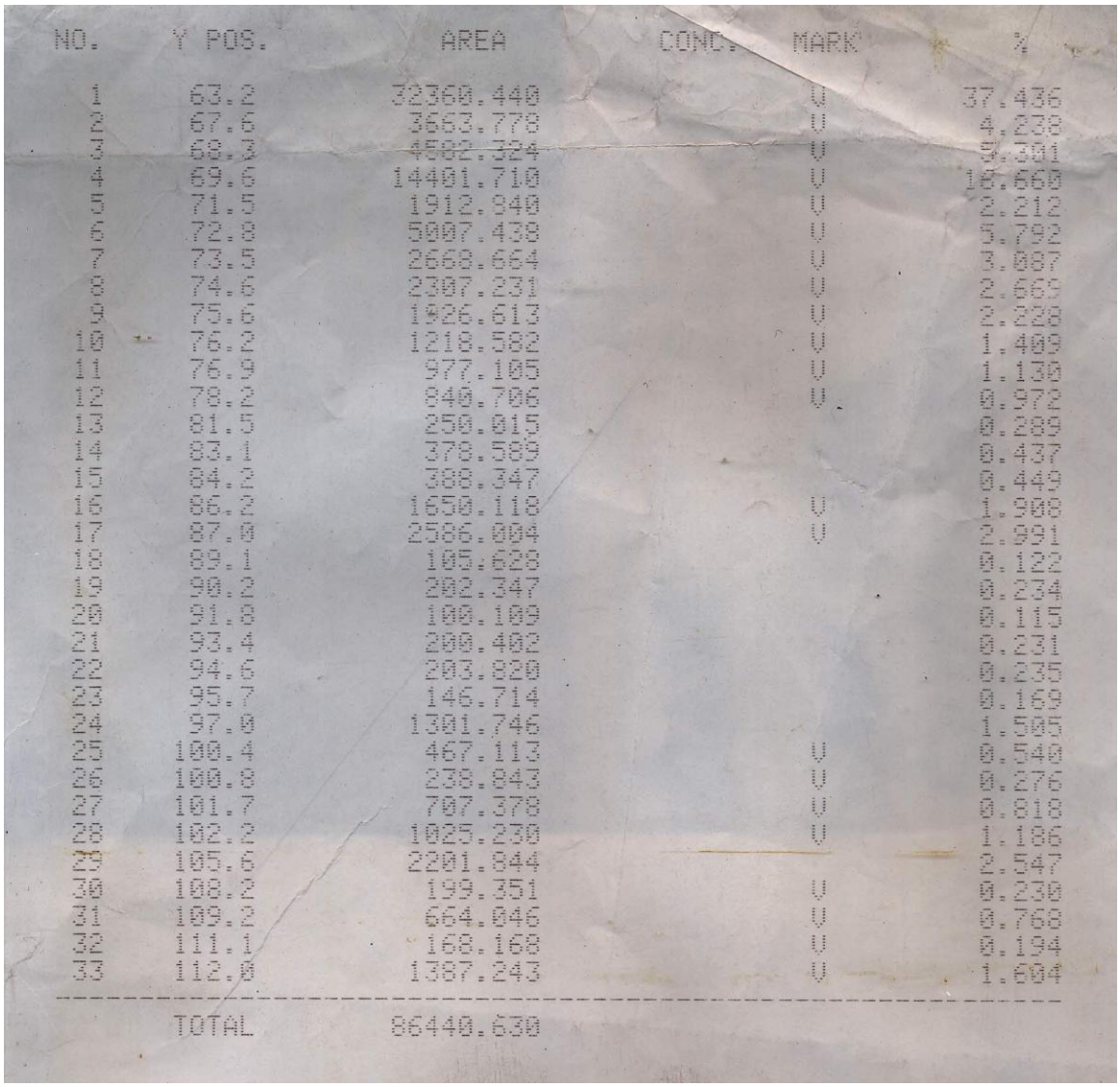
Appendix. 9 Purple stemmed morphotype glucosinolates analysis



NO.	Y POS.	AREA	CONC.	MARK	%
1	64.9	1169.816			1.769
2	66.0	9202.669		V	13.928
3	67.6	2062.856		V	3.120
4	71.0	2567.156			3.883
5	75.9	488.636		V	0.739
6	76.5	410.492		V	0.621
7	77.2	613.410		V	0.928
8	78.0	131.808			0.199
9	79.7	144.929		V	0.219
10	81.8	3543.430		V	5.360
11	83.3	4059.473		V	6.141
12	84.5	1008.480		V	1.525
13	86.9	149.265			0.225
14	89.0	144.839			0.219
15	90.6	718.726			1.087
16	91.5	109.566			0.165
17	93.0	195.820			0.296
18	93.9	202.078			0.305
19	95.4	481.859			0.729
20	96.6	171.601			0.259
21	98.1	287.910			0.435
22	99.7	211.546			0.320
23	101.5	228.941			0.346
24	102.4	249.382			0.377
25	104.0	184.222			0.278
26	105.0	187.527		V	0.283
27	106.3	617.293		V	0.933
28	107.3	482.937			0.730
29	109.0	110.425			0.167
30	110.8	1002.855			1.517
31	112.3	639.019			0.966
32	117.2	28959.450		V	43.813
33	120.4	5358.535		V	8.107
	TOTAL	66096.940			

Highest glucosinolates- peak at 43 %

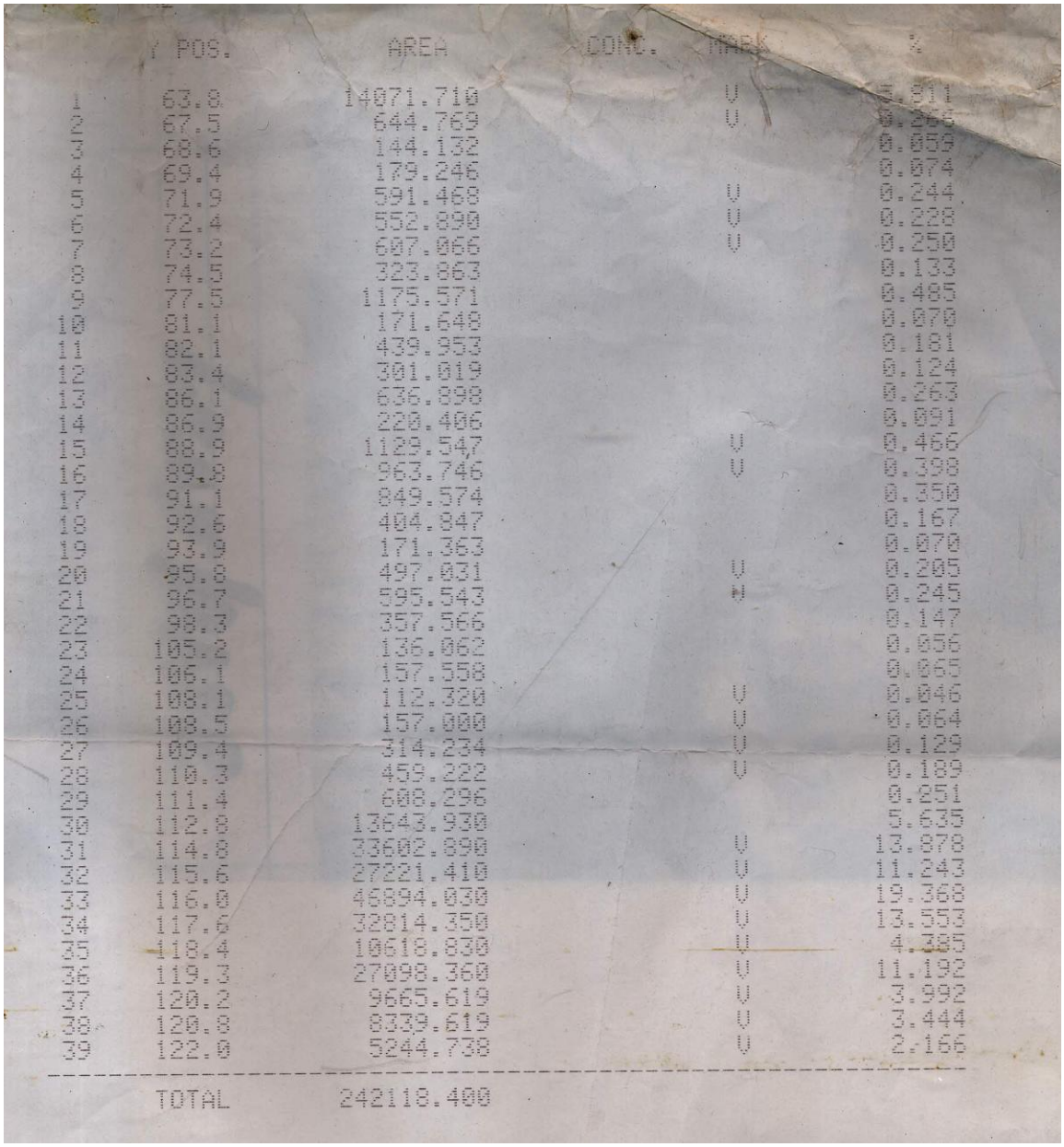
Appendix. 10 Purple-green stemmed morphotype glucosinolates analysis



NO.	Y POS.	AREA	CONC.	MARK	%
1	63.2	32360.440		V	37.436
2	67.6	3663.778		V	4.238
3	68.3	4502.324		V	5.301
4	69.6	14401.710		V	16.660
5	71.5	1912.840		V	2.212
6	72.8	5007.438		V	5.792
7	73.5	2668.664		V	3.087
8	74.6	2307.231		V	2.669
9	75.6	1926.613		V	2.228
10	76.2	1218.582		V	1.409
11	76.9	977.105		V	1.130
12	78.2	840.706		V	0.972
13	81.5	250.015			0.289
14	83.1	378.589			0.437
15	84.2	388.347			0.449
16	86.2	1650.118		V	1.908
17	87.0	2586.004		V	2.991
18	89.1	105.628			0.122
19	90.2	202.347			0.234
20	91.8	100.109			0.115
21	93.4	200.402			0.231
22	94.6	203.820			0.235
23	95.7	146.714			0.169
24	97.0	1301.746			1.505
25	100.4	467.113		V	0.540
26	100.8	238.843		V	0.276
27	101.7	707.378		V	0.818
28	102.2	1025.230		V	1.186
29	105.6	2201.844			2.547
30	108.2	199.351		V	0.230
31	109.2	664.046		V	0.768
32	111.1	168.168		V	0.194
33	112.0	1387.243		V	1.604
	TOTAL	86440.630			

Highest glucosinolates- peak at 37 %

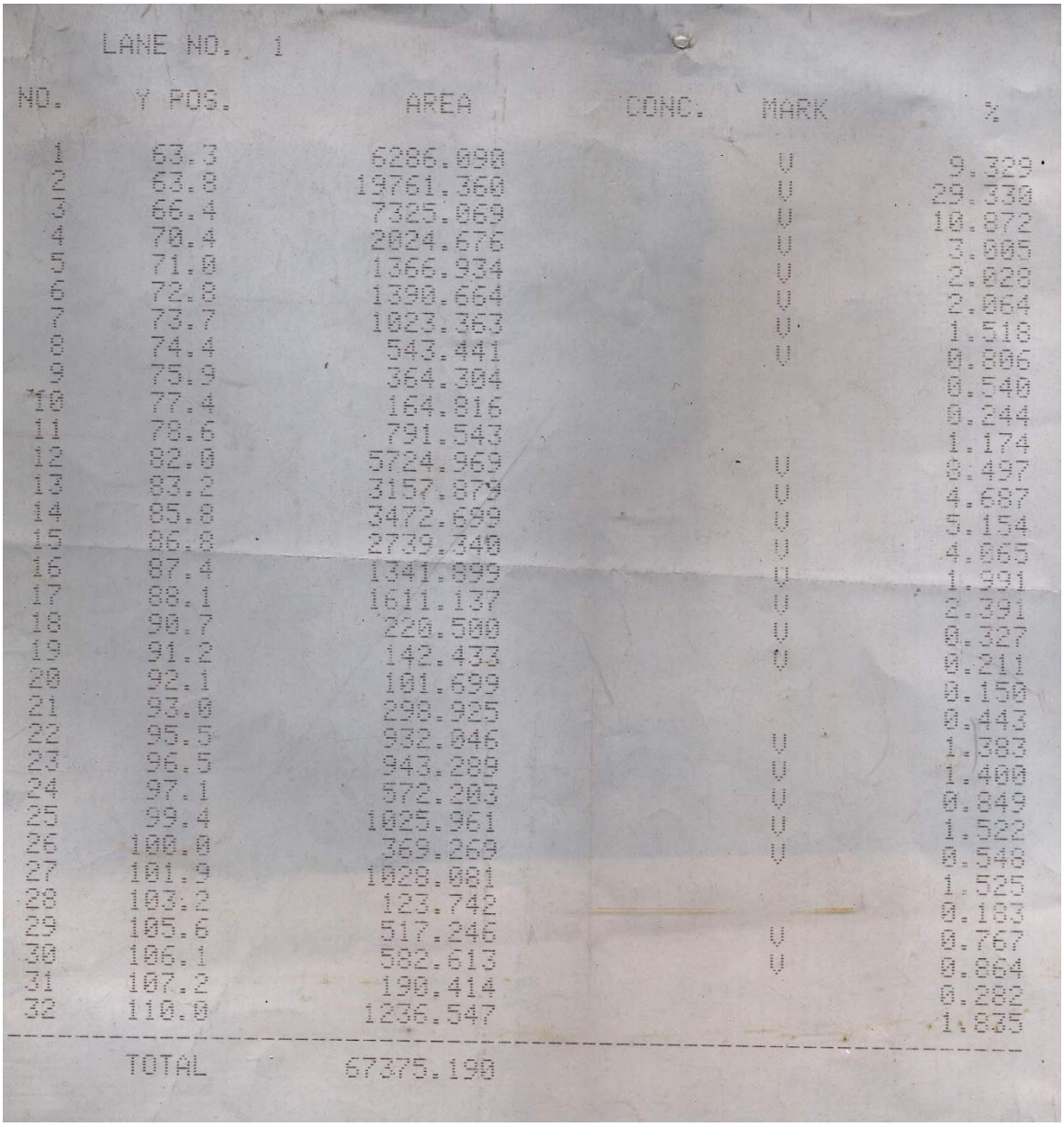
Appendix. 12 Green stemmed morphotype glucosinolates analysis



	R POS.	AREA	CONC.	MARK	%
1	63.8	14071.710		V	5.811
2	67.5	644.769		V	0.266
3	68.6	144.132			0.059
4	69.4	179.246			0.074
5	71.9	591.468		V	0.244
6	72.4	552.890		V	0.228
7	73.2	607.066		V	0.250
8	74.5	323.863			0.133
9	77.5	1175.571			0.485
10	81.1	171.648			0.070
11	82.1	439.953			0.181
12	83.4	301.019			0.124
13	86.1	636.898			0.263
14	86.9	220.406			0.091
15	88.9	1129.547		V	0.466
16	89.8	963.746		V	0.398
17	91.1	849.574			0.350
18	92.6	404.947			0.167
19	93.9	171.363			0.070
20	95.8	497.031		V	0.205
21	96.7	595.543		V	0.245
22	98.3	357.566			0.147
23	105.2	136.062			0.056
24	106.1	157.558			0.065
25	108.1	112.320		V	0.046
26	108.5	157.000		V	0.064
27	109.4	314.234		V	0.129
28	110.3	459.222		V	0.189
29	111.4	608.296			0.251
30	112.8	13643.930			5.635
31	114.8	33602.890		V	13.878
32	115.6	27221.410		V	11.243
33	116.0	46894.030		V	19.368
34	117.6	32814.350		V	13.553
35	118.4	10618.830		V	4.385
36	119.3	27098.360		V	11.192
37	120.2	9665.619		V	3.992
38	120.8	8339.619		V	3.444
39	122.0	5244.738		V	2.166
	TOTAL	242118.400			

Highest glucosinolates- peak at 19 %

Appendix. 12 Purple stemmed morphotype terpenoids analysis

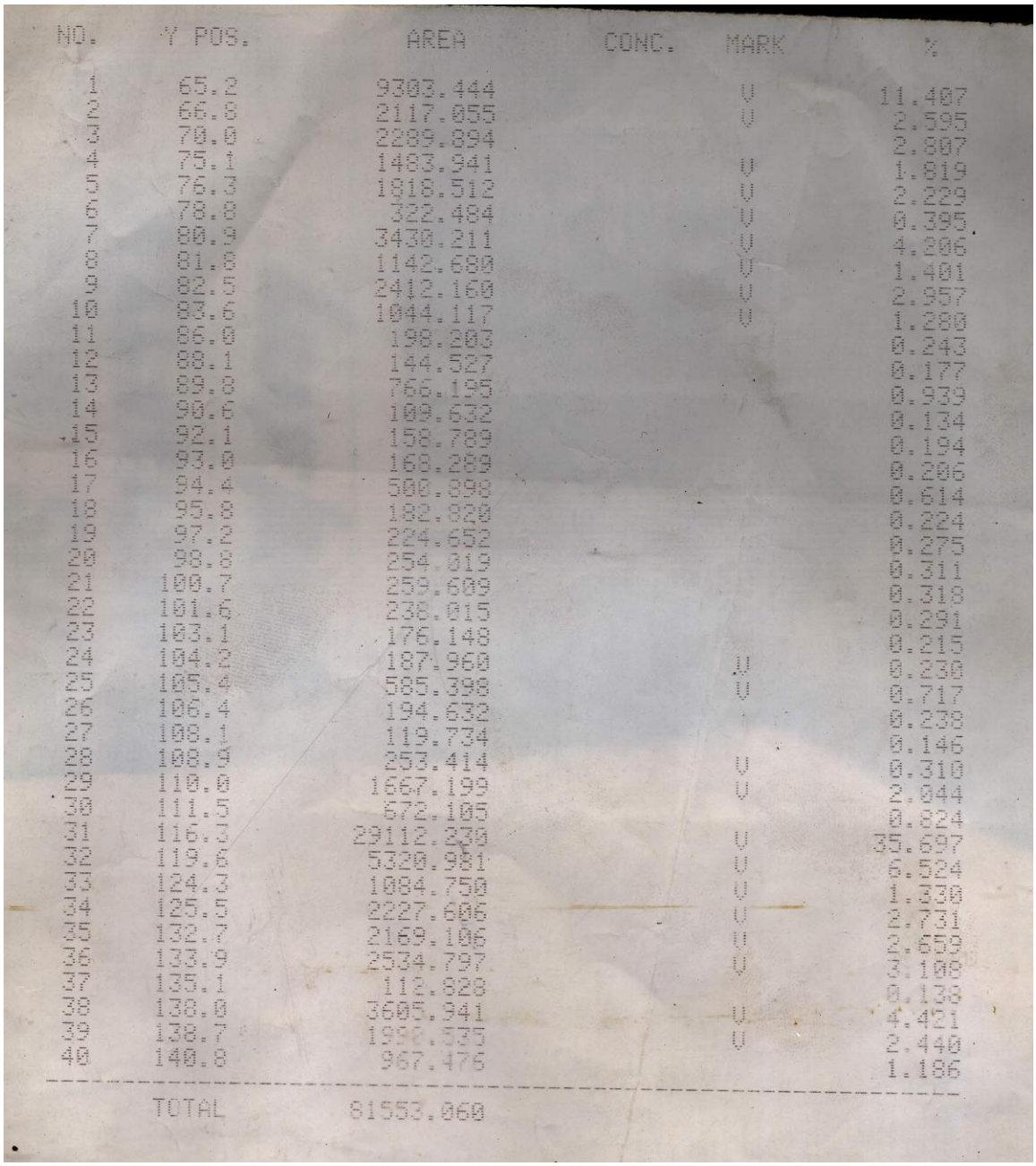


LANE NO. 1

NO.	Y POS.	AREA	CONC.	MARK	%
1	63.3	6286.090		V	9.329
2	63.8	19761.360		V	29.330
3	66.4	7325.069		V	10.872
4	70.4	2024.676		V	3.005
5	71.0	1366.934		V	2.029
6	72.8	1390.664		V	2.064
7	73.7	1023.363		V	1.518
8	74.4	543.441		V	0.806
9	75.9	364.304			0.540
10	77.4	164.816			0.244
11	78.6	791.543			1.174
12	82.0	5724.969		V	8.497
13	83.2	3157.879		V	4.687
14	85.8	3472.699		V	5.154
15	86.8	2739.340		V	4.065
16	87.4	1341.899		V	1.991
17	88.1	1611.137		V	2.391
18	90.7	220.500		V	0.327
19	91.2	142.433		V	0.211
20	92.1	101.699			0.150
21	93.0	298.925			0.443
22	95.5	932.046		V	1.383
23	96.5	943.289		V	1.400
24	97.1	572.203		V	0.849
25	99.4	1025.961		V	1.522
26	100.0	369.269		V	0.548
27	101.9	1028.081			1.525
28	103.2	123.742			0.183
29	105.6	517.246		V	0.767
30	106.1	582.613		V	0.864
31	107.2	190.414			0.282
32	110.0	1236.547			1.835
	TOTAL	67375.190			

Highest terpenoids- peak at 29 %

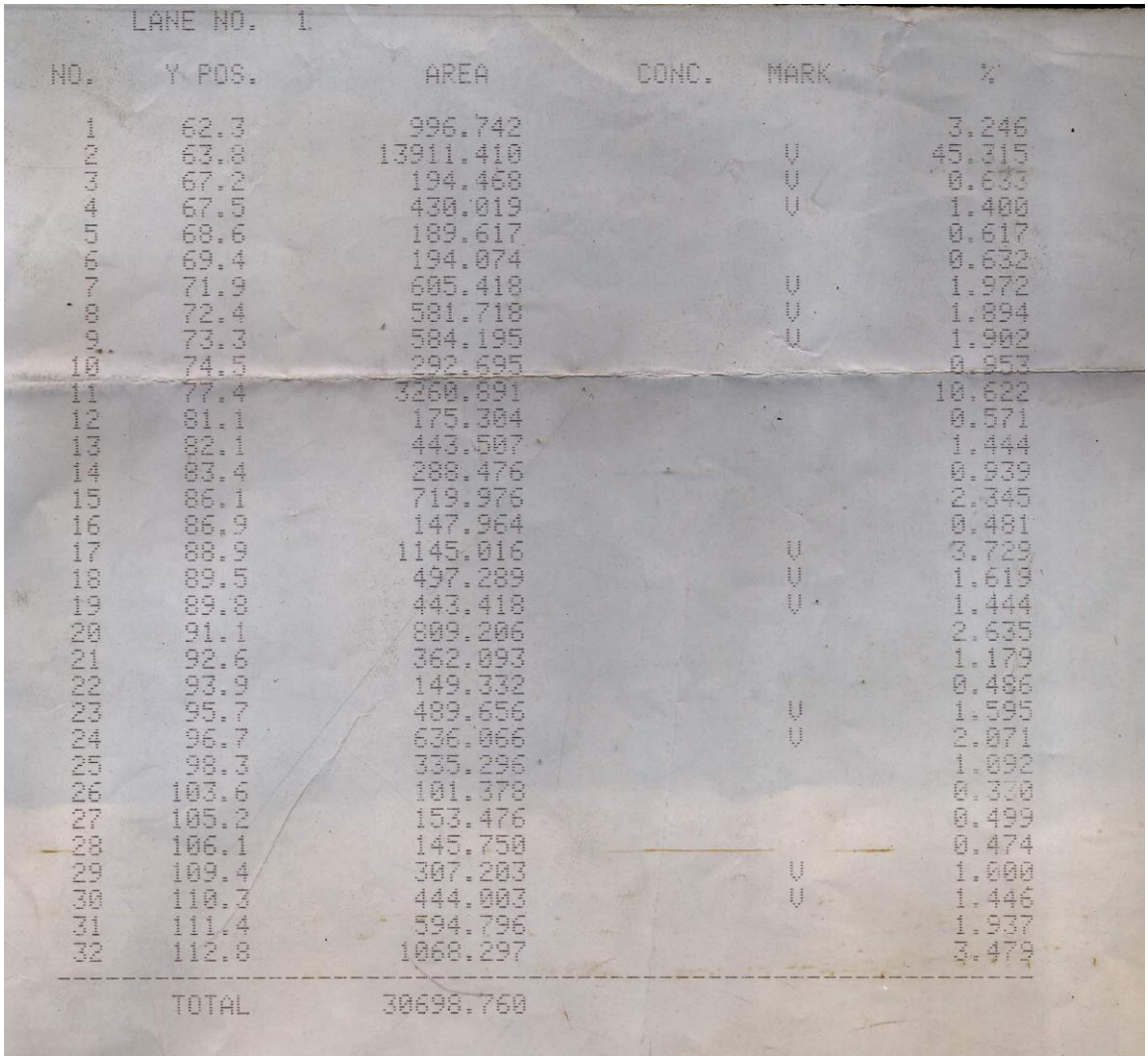
Appendix. 13 Purple-green stemmed morphotype terpenoids analysis



NO.	Y POS.	AREA	CONC.	MARK	%
1	65.2	9303.444		V	11.407
2	66.8	2117.055		V	2.595
3	70.0	2289.894			2.807
4	75.1	1483.941		V	1.819
5	76.3	1818.512		V	2.229
6	78.8	322.484		V	0.395
7	80.9	3430.211		V	4.206
8	81.8	1142.680		V	1.401
9	82.5	2412.160		V	2.957
10	83.6	1044.117		V	1.280
11	86.0	198.203			0.243
12	88.1	144.527			0.177
13	89.8	766.195			0.939
14	90.6	109.632			0.134
15	92.1	158.789			0.194
16	93.0	168.289			0.206
17	94.4	500.898			0.614
18	95.8	182.820			0.224
19	97.2	224.652			0.275
20	98.8	254.819			0.311
21	100.7	259.609			0.318
22	101.6	238.015			0.291
23	103.1	176.148			0.215
24	104.2	187.960		V	0.230
25	105.4	585.398		V	0.717
26	106.4	194.632			0.238
27	108.1	119.734			0.146
28	108.9	253.414		V	0.310
29	110.0	1667.199		V	2.044
30	111.5	672.105			0.824
31	116.3	29112.330		V	35.697
32	119.6	5320.981		V	6.524
33	124.3	1084.750		V	1.330
34	125.5	2227.606		V	2.731
35	132.7	2169.106		V	2.659
36	133.9	2534.797		V	3.108
37	135.1	112.828			0.138
38	138.0	3605.941		V	4.421
39	138.7	1990.535		V	2.440
40	140.8	967.476			1.186
	TOTAL	81553.060			

Highest terpenoids- peak at 35 %

Appendix. 14 Green stemmed morphotype terpenoids analysis



LANE NO. 1

NO.	Y POS.	AREA	CONC.	MARK	%
1	62.3	996.742			3.246
2	63.8	13911.410		V	45.315
3	67.2	194.468		V	0.633
4	67.5	430.019		V	1.400
5	68.6	189.617			0.617
6	69.4	194.074			0.632
7	71.9	605.418		V	1.972
8	72.4	581.718		V	1.894
9	73.3	584.195		V	1.902
10	74.5	292.695			0.953
11	77.4	3260.891			10.622
12	81.1	175.304			0.571
13	82.1	443.507			1.444
14	83.4	288.476			0.939
15	86.1	719.976			2.345
16	86.9	147.964			0.481
17	88.9	1145.016		V	3.729
18	89.5	497.289		V	1.619
19	89.8	443.418		V	1.444
20	91.1	809.206			2.635
21	92.6	362.093			1.179
22	93.9	149.332			0.486
23	95.7	489.656		V	1.595
24	96.7	636.066		V	2.071
25	98.3	335.296			1.092
26	103.6	101.378			0.330
27	105.2	153.476			0.499
28	106.1	145.750			0.474
29	109.4	307.203		V	1.000
30	110.3	444.003		V	1.446
31	111.4	594.796			1.937
32	112.8	1068.297			3.479
	TOTAL	30698.760			

Highest terpenoids- peak at 45 %