

DECLARATION

Declaration by the student

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DEDICATION

To my beloved mother R. Ashiali, my beloved wife Esther N. Ketiba, my daughters Linet, Joslet, Mercy and Sylvia, my sons Josphat, Elvis and Gideon who supported and encouraged me during this academic journey.

ABSTRACT

The black nightshade, *Solanum nigrum* Linnaeus (Solanaceae), is a widely distributed tropical plant used as a nutritive vegetable and herbal medicine in East Africa. This plant expresses high levels of secondary metabolites such as steroidal glycoalkaloids (SGA) and phytoalexins, which offer protection against pests and microbial pathogens. However, insect pests especially the black bean aphid *Aphis fabae* Linnaeus (Homoptera, Aphididae) and the red spider mite *Tetranychus evansi* Linnaeus (Acarina, Tetranychidae), have become a major problem for *S. nigrum* Linnaeus, especially the improved cultivars with less bitterness that are being adopted in western Kenya. The effects of *A. fabae* Linnaeus are aggravated by attendant ants that protect them while cleaning their sugary secretions. The current research evaluated the potential of onion (*Allium cepa* Linnaeus) extracts and farmyard manures in the integrated control of *A. fabae* Linnaeus and *T. evansi* Linnaeus infesting three *S. nigrum* Linnaeus cultivars in western Kenya. The field study was conducted within Masinde Muliro University of Science and Technology farm, in Kakamega County, western Kenya. The experiment was a randomized block design of 8 blocks of 3.6 m x 4 m with 9 plots represented by rows of 20 plants. Two cultivars of *S. nigrum* var. *scabrum* Linnaeus and one cultivar of *S. nigrum* var. *nigrum* Linnaeus were used. Extracts were prepared using a Detergent water method. Cattle and chicken manure were obtained from the University farm and left to mature and dry naturally in a shade before being crushed to fine particles. Field investigations included pest population build up, pest enumeration, plant height, leaf deformations and biomass. Sampling was done once every week on plants in situ one day after spraying with the onion extracts and numbers counted. Data was analyzed using SAS version 9.1. The number of aphids was highest in cultivar B (*Solanum nigrum* var. *nigrum* Linnaeus). Plants grown with cattle manure grew better than those grown with chicken manure or without manure. Pest populations were high in plants grown with chicken manure, especially cultivar B, showing a preference that occurs during the presence of non-preferred hosts. Four attendant ant species, *Dolichoderus mariae*, *Formica glarialis*, *Formica padelifulva* and *Camponotus sp* protected aphids from predators. Application of onion extracts reduced pest populations, and cultivars F and M tended to have the best effect.

ACKNOWLEDGEMENT

I am highly indebted to my supervisors Prof. F.M.E. Wanjala and Dr. Dennis M.W. Ochieno for their academic and technical guidance during this study. Special thanks go to Dr. Paul Wanjala, Prof. Elizabeth Njenga, Prof. Ezekiel Kiprop, Prof. Donald Otieno, Prof. William Shivoga, Prof. Thomas Sakwa, Dr. Millicent Ndong'a, Dr. John Muoma, and Dr. Stella Kirui for their efforts that helped me to overcome academic challenges during this study. Prof. Phillip Wandahwa of the Faculty of Agriculture and Veterinary Sciences, Masinde Muliro University of Science and Technology availed the plot on which the experiments were conducted. I appreciate the relentless assistance by Victoria Naluyange during the data analysis and interpretation. I recognize the technical support from Fredrick Wandera, Eric Bushuru and Simon Kasili. Finally I am thankful to my technical colleagues in the Department of Biological Sciences, Peter Nyongesa, Aggrey Osogo, Nicholas Kitungulu, Willy Aganyanya, Grace Olwenyo, Richard Wepukhulu, Consolata Shilaho and Kefa Ogenche and all academic staff for their academic and technical advises.

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ACRONYMS AND ABBREVIATIONS

A.s.l	Above sea level
ANOVA	Analysis of variance
FORMAT	Forum for organic resource management and technologies
IPM	Integrated Pest Management
MMUST	Masinde Muliro University of Science and Technology
SGA	Steroidal glycoalkaloids

CHAPTER ONE

INTRODUCTION

1.1 General introduction

The black nightshade, *Solanum nigrum* Linnaeus. (Solanaceae), is a widely distributed tropical plant used as a nutritive vegetable and herbal medicine in East Africa (Edmonds and Chweya, 1997; Adebooye and Opabole, 2004; Chandrashekhar *et al.*, 2013). Black nightshade is highly adaptable to local growing conditions, requires low production inputs while exhibiting fast growth and extended harvesting period (Schippers, 2000). *S. nigrum* Linnaeus expresses high levels of secondary metabolites such as steroidal glycoalkaloids (SGA) and phytoalexins, which offer protection against pests and microbial pathogens (Hammond-Kosack *et al.*, 2003; Thatcher *et al.*, 2005). Therefore, farmers in East Africa do not apply synthetic pesticides on this vegetable. This has made the crop preferable amidst the high demand for organic vegetables that are not contaminated with toxic synthetic pesticides (Magkos *et al.*, 2006; Blair and Robert, 2012). Although the enhanced expression of pest-inhibitive compounds in black nightshade is desirable, the bitter taste of such compounds has been a hindrance to their acceptance in the market. Therefore, plant breeders and biotechnologists have been developing improved cultivars that have less bitterness (Cheatle *et al.*, 1993). However, such improved less bitter cultivars express low levels of pest-inhibitive compounds, and hence exhibit reduced resistance to pests and pathogens (Sanford *et al.*, 1992). Currently, arthropod pests such as the black bean aphid *Aphis fabae* (Homoptera, Aphididae) and the red spider mite *Tetranychus evansi* Linnaeus (Acarina, Tetranychidae) have become a

major problem for improved *S. nigrum* Linnaeus cultivars, especially in regions such as western Kenya where the varieties are being adopted. Development of organic solutions to pests and soil fertility problems in the cultivation of improved varieties of *S. nigrum* Linnaeus is therefore necessary. The current research aimed at developing an integrated pest management (IPM) approach based on *A. cepa* Linnaeus extracts and matured farmyard manures to control the aphid *A. fabae* Linnaeus and a mite *T. evansi* Linnaeus infesting improved three *S. nigrum* Linnaeus cultivars in western Kenya.

1.2 Statement of the problem

Low levels of bitter compounds in improved *S. nigrum* Linnaeus cultivars have led to reduction in resistance to pests (Sanford *et al.*, 1992; Yi *et al.* 2012). Farmers have been reverting to the use of inorganic fertilizers and synthetic pesticides to complement each other in controlling aphids and mites (Marschner, 1995). Synthetic pesticides have been reducing in efficacy due to resistance development in aphids and mites globally (Herron *et al.*, 2004a; 2005), while inorganic fertilizers are expensive, besides being linked to soil and water pollution (Naluyange *et al.*, 2014). Plants supplied with nutrient from biological materials are more resistant to insects than those grown using chemical fertilizers (Raja, 2012). Application of pesticides from plant extracts keeps aphids under control (Mochiah *et al.*, 2011).

1.3 Justification of the study

The use of inorganic pesticides and fertilizers on vegetables is associated with pest resurgence and threats on human health and the environment, besides their high costs. Nightshades (*S. nigrum* Linnaeus) are on high demands in western Kenya due to their high nutritional and medicinal values (Gohole *et al.*, 2013). This integrated pest

management system is cheap and safe for the health of the vegetable, humans and the environment.

1.4 Objectives

The general objective of this study was to evaluate the integration of aqueous extracts from the onion, *A. cepa* Linnaeus and dry farmyard manures for the control of the arthropod pests infesting *S. nigrum* Linnaeus cultivars under field conditions in western Kenya.

Specific objectives of the study were:-

1. To determine the susceptibility of three cultivars of *S. nigrum* Linnaeus to infestation and damage by *A. fabae* Linnaeus and *T. evansi* Linnaeus.
2. To evaluate the efficacy of *Allium cepa* Linnaeus extracts on *A. fabae* Linnaeus and *T. evansi* Linnaeus when planted on *S. nigrum* Linnaeus cultivars when planted with different manuring regimes.
3. To evaluate efficacy of *Allium cepa* Linnaeus aqueous extracts on non-target attendant ants associated with aphids (*A. fabae* Linnaeus) infestation on *S. nigrum* Linnaeus.

1.5 Hypotheses

1. H₀. *S. nigrum* Linnaeus cultivars vary in their biological attributes that make them have different levels of susceptibility to aphids and spider mites.
2. H₀. Combined application of onion extracts and farmyard manures on pest-susceptible *S. nigrum* Linnaeus suppresses aphid and spider mite infestations while increasing vegetable growth.

3. H₀. Application of farmyard manures and onion extracts changes the function of *S. nigrum* Linnaeus plant in a way that reduces symbiotic ants that favor infestations by aphids.

CHAPTER TWO

LITERATURE REVIEW

2.1. Black nightshade *Solanum nigrum* Linnaeus

Black nightshade is a complex of six solanaceous herbs namely *S. nigrum*, *S. gracile*, *S. villosum*, *S. nodiflorum* (*S. americanum*), *S. retroflexum* and *S. scabrum* (Edmonds and Chweya, 1997). They are consumed as leafy vegetables, are sources of indigenous fruits and for medicinal purposes (Edmonds and Chweya, 1997; Okole and Adhav, 2004; Adebooye and Opabole, 2004). *S. nigrum* species are among the most common and highly important leafy vegetables in the warm and humid zones of Africa (Schippers, 2002).

2.2 Arthropod pests of black nightshades and other solanaceous plants

Two groups of arthropods, namely class insecta and class arachnida attack black nightshades and other solanaceous plants (Brisson and Davis, 2008). Insect pests that attack nightshades and other solanaceous plants belong to the orders homoptera in families Aphididae and Cicadellidae, Coleopteran family Chrysomellidae, one Orthopteran family Acrididae and one Thysanopteran family, Thripidae. Aphids, in the order homoptera, are the leading problem on solanaceous plants (Foster and John 2010; Brisson and Davis, 2008). They cause mottled leaves, yellowing and stunted growth, curled leaves, browning and reduced yields on the host plants (McGavin *et al.* 1993). In Kenya, the most common aphids found infesting the black nightshades are the black bean aphid, *A. fabae* and the green peach aphid, *Myzus persicae*. The aphids have been found to transmit viral and fungal plant diseases. For example, black bean aphids, *A. fabae* has been found to transmit the late blight, *Phytophthora infestans* on

potatoes while the green peach aphids, *M. persicae* transmit plant viruses on solanaceous plants (Nicholas, 2007; Marava, 2012). The production of honeydew that forms a coating on the leaf surface has been reported to promote fungal growth and also reduces the effectiveness of fungicides (Gillman, 2005; Reynolds and Volk, 2007; Dik and Pelt, 1992).

Solanaceous plants are also infested by spider mites (Acarina, Tetranychidae), which include the spotted spider mite, *Tetranychus urticae* and the red spider mite, *T. evansi*, with the red spider mite being reported as the prominent problem on the black nightshades (Brisson and Davis, 2008). The red spider mite is a polyphagous mite and feeds on wild and cultivated solanaceae, especially tomatoes, potatoes, black nightshades and tobacco (MacLeod 2008). The pest also feeds on a number of non-solanaceous plants like beans, citrus, cotton, tea, coffee and castor beans (MacLeod, 2008). Damage by red spider mites causes leaves to become chlorotic with the formation of white to brown spots twisted leaves and low productivity (FORMAT, 2003). Red spider mites are identified by formation of spider webs and formation of white to brown spots.

2.2.1 Aphid pests and their symbionts

The success of aphids in causing heavy infestations on black nightshades and other solanaceous plants partly depends on the mutualistic relationship with other insects (Linda and Hooper 2008). Aphids form mutualistic associations with black ants e.g. Argentine ants, *Linepithema humile* (Formicidae) (Crystal *et al.*, 2008). In such associations, the ants feed on honey dew secreted by aphids, while they in turn provide protection for the aphids against natural enemies as well as cleaning the aphid environment (Crystal *et al.*, 2008). Ants that co-exist mutualistically with aphids

exhibit fitness in terms of abundance, activity, and levels of predation on other arthropods (Kaplan and Eubanks, 2005).

2.2.2 Aphid defenses and adaptations

Some galling aphid species have been reported to produce specialized soldier forms, which defend the galls from invasion by predators. Cornicles (siphunculi) have been found to produce defensive chemicals such as waxes (Kazana *et al* 2007).

2.3 Solanaceous plant resistance against arthropod pests

Plants have mechanisms of defending themselves against infestation and damage by pests and pathogens (Gregg *et al.*, 2008). There are three categories of mechanisms by which plants may accomplish this, antixenosis, antibiosis and tolerance. Antixenosis is a mechanism, by which a plant either fails to serve as a host to the pest, or the pest prefers an alternative host (Smith, 2005) while in antibiosis, pest physiology is affected, precipitating in adverse effects on growth, development, reproduction and/or survival of the pest (Smith, 2005). This may result from the production of secondary metabolites for the plant defense (Gatehouse, 2002). Tolerance is the ability of the plant to withstand and/or recover from damage caused by a pest at a scale that is comparable to that on a plant with-out any resistant characteristics (Pegido, 1999). Solanaceous plants have developed ways of protecting themselves and have been found to have varying levels of resistance to pests (Stout *et al.*, 1994; Frechette *et al.*, 2010). *S. nigrum* cultivars produce metabolites such as steroidal glycoalkaloids and phytoalexins, which inhibit *A. fabae* infestations and damage (Frechette *et al* 2010; Ashilenje *et al.*, 2011). In a comparative study, Nelson *et al.* (2013) found that *Solanum lycopersicum* was more susceptible to the tomato fruit

borer *Neoleucinodes elegantalis* compared to other three wild *Solanum* species i.e. *Solanum habrochaites* var. *glabrutum* and *Solanum habrochaites* var. *typicum*.

2.3.1. Role of plant nutrition in plant defense against pests

Plants require three categories of mineral nutrients for growth and development namely, macronutrients, secondary nutrients and micronutrients (Table 2.1). Macronutrients (6-100%) are the nutrients which are required in large quantities and include nitrogen (N), potassium (K) and phosphorus (P) while secondary nutrients (3-12.5%) are those that are required in medium quantities and consist of calcium (Ca), magnesium (Mg) and sulphur (S). Micronutrients (0.0001-0.3%) are those nutrients that are required in minute quantities, and include iron (Fe), manganese (Mn), zinc (Zn), boron (B), copper (Cu) chlorine (Cl) and molybdenum (Table 2.1) (Timothy *et al.*, 2013).

Timothy *et al.* (2013) also reported that imbalances in these nutrients increase susceptibility of plants to pests and pathogenic infections. High soluble N levels in plant tissues have been associated with decrease in plant resistance against insect pests and diseases (Miguel *et al.*, 2003). Excessive use of chemical fertilizers creates nutrient imbalances in plants, which in turn, reduce resistance to arthropod pests (Timothy *et al.*, 2013). Organic soil fertility amendments provide secondary and trace elements that stimulate resistance to insect attack (Patriquin *et al.*, 1995; Altieri *et al.*, 2003; Chau *et al.*, 2005); Sarwar, 2012). Organic N sources have been found to allow greater tolerance to vegetative damage by insect pests in plants because such sources release N more slowly (Timothy *et al.*, 2010). Plants deficient in potassium are highly susceptible to pest infestation and damage (Amtmann *et al.*, 2008).

Table 2.1 Essential mineral nutrients and their functions in plant growth and development (Source: Timothy *et al.*, 2013)

	Nutrient	Chemical symbol	Relative abundance (%)	Function in plant
Macronutrients	Nitrogen	N	100	Proteins, amino acids
	Potassium	K	25	Catalyst, iron transport
	Phosphorus	P	8	Nucleic acid, ATP
Secondary nutrients	Calcium	Ca	12.5	Cell wall component
	Magnesium	Mg	8	Part of chlorophyll
	Sulphur	S	3	Amino acids
Micronutrients	Iron	Fe	0.2	Chlorophyll synthesis
	Manganese	Mn	0.1	Activates enzymes
	Chlorine	Cl	0.3	Photosynthesis reaction
	Boron	B	0.2	Cell wall component
	Copper	Cu	0.01	Component of enzymes
	Zinc	Zn	0.03	Activates enzymes
	Molybdenum	Mo	0.0001	Involved in N fixation

2.4 Arthropod evasion of plant defenses

Aphids evade plant defenses by making their stylets exert intercellular penetrations that avoid cell puncturing (Tjallingii, 1990; Walling, 2000) while obtaining nutrients from the vascular tissue (Blackman and Eastop, 2000). The spider mite *T. evansi* down-regulates the production of plant defensive compounds such as proteinase inhibitors within host leaves (Sarmiento *et al.*, 2011). It also produces a silken web over the leaves they feed on as a defense against their predators.

2.5 Botanical pesticides for aphid and spider mite control

Botanical extracts from a number of flowering plants have been screened and found to be effective in the control of a pest complex on *S. nigrum*. A vast majority of plant extracts require to be dissolved in water or soap prior to application. Prassanath and Mahendran (2013) reported that neem, *Azadirachta indica* (Meliaceae) extracts have been used in the control of aphids, leaf miners and pod borers in cow peas. Extracts from chinaberry tree, *Melia azedarach* (Meliaceae) have also been found to be effective in the control of the cabbage aphid *Brevicoryne brassicae* (Nagappan, 2012). Sprays from pyrethrum, *Chrysanthemum cinerariaefolium* have been successful in the control of ants and aphids; while sprays made from the castor oil plant have been used on many other pests (Akhtar *et al.*, 2007).

Onions (*Allium cepa*) contain compounds of sulphur and allinin that have been linked with the defense against pests and microbial pathogens (Grubb and Abel, 2006; Halkier and Gershenzon, 2006). Sulphoxides, which include the lachrymatory factor in onion and allicin in garlic, have molluscicidal and insecticidal properties (Amault *et al.*, 2008).

Alliinase, an enzyme from onions is defensive and has been reported to be lethal to a wide range of pests (Vijayalakshmi *et al.*, 1999; Stoll 2000). This is why the effects of onion were investigated in this study.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

A field study was conducted within the facilities of Masinde Muliro University of Science and Technology farm, in Kakamega County, western Kenya (latitude N 00 17.104', longitude E 034⁰ 45.874'; altitude 1561m a.s.l.) (Naluyange *et al.*, 2014). The study area has two rain seasons, the long rain season (April – August) and the short rain season (September – December). Annual rainfall is ~1,800 mm; with an average annual temperature of 20.8°C (Naluyange *et al.*, 2014). Soils in this area are loamy with the properties described in Table 3.1.

3.2 Experimental design

The experiment was a randomized block design on a field (20m x 10m), divided into 8 blocks of (3.6m x 4m), each comprising of 9 plots in the form of lines of 20 planting holes, that were spaced at 40 cm x 20 cm. The treatments were factorial (3 x 3 x 2) with soil fertility factor (cattle manure, chicken manure, or without manure), plant variety factor (*S. nigrum var. nigrum*, *S. nigrumvar. scabrum* collection A, and *S. nigrumvar. scabrum* collection B), and the botanical spray factor (onion extracts or water). This resulted in 18 treatment combinations with n=20 plants. The experiment was conducted in the year 2012, with the first trial between April and August, and repeated between September and December.

3.3 Planting material

Seeds of *S. nigrum var. nigrum* (Simlaw Seeds, Kenya Seed Company Ltd, Kitale, Kenya) were purchased from an agro-vet shop in Kakamega town. The seeds are whitish

cream, approximately 1mm in diameter. The ripe fruit is maroon in color and approximately 10mm in diameter. The plants have leaves that are pointed and longer than wide, with internodes of approximately 5cm (Plate 3.2). *S. nigrum* var. *scabrum* (A) seeds were obtained from the MMUST farm. This cultivar has seeds light to dark brown in color and approximately 2mm in diameter. The ripe fruit is maroon in color and measures about 20mm in diameter. The leaves are bright green and either wider than long or are wide as long in appearance with the internodes of about 4cm (Plate 3.1). Seeds of *S. nigrum* var. *scabrum* (B) were purchased from the Kakamega open market. The cultivar has characteristics like those of the cultivar *S. nigrum* var. *scabrun* (A), except the leaves are deep green.



Plate 3.1 A close up of a healthy *S.nigrum* var *scabrum* during field trials
(Source: Author, 2012)



Plate 3.2: A mature *S. nigrum* var *nigrum*

3.4 Biopesticide and manure

The biopesticide comprised of extracts made from *A. cepa* purchased from the Kakamega open market; the biopesticide was prepared using the detergent-water method (Vijayalakshmi *et al.*, 1999), at the Laboratory of Biotechnology (MMUST). Portions of

chopped and crushed onions (85 g) were added to vegetable oil (50mL) (Golden Fry, BIDCO Kenya Ltd, Nairobi). The mixture was allowed to stand for 24 hours, then 1 mL of liquid detergent (Ushindi liquid detergent (Cussons Kenya Ltd, Nairobi, Kenya) and 950mL of tap water was added, followed by maceration using kitchen blender (Philips; China) and straining of the mixture using a sieve (0.5mm mesh size; Ken Poly, Nairobi, Kenya). The mixture was used in the experiment on the day of preparation. Chicken and cattle matured manures were obtained from Masinde Muliro University of Science and Technology farm. The manures were dried in the shade and crushed into fine particles.

Table 3.1. Experimental plot soil composition. (Source: Naluyange *et al.* 2014).

Nutrient	Concentration	Units
Organic carbon	2.5	Percentage
Total nitrogen	0.25	Percentage
Total phosphorus	18.9	ppm
Potassium	0.41	cmolc kg-1
Sodium	0.1	cmolc kg-1
Calcium	2.3	cmolc kg-1
Magnesium	0.8	cmolc kg-1
Zinc	1.9	ppm
Iron	0.37	ppm
pH	4.2	

3.5 Planting and spraying

During planting, a table spoonful of manure was thoroughly mixed with the topsoil in each planting hole, as per the respective manure treatments. One seed was sown per hole at a depth of about 2mm, and covered with a thin layer of top soil. The plots were rain-fed and therefore no additional water was applied. Weeding was done every 2 weeks using a hoe. Spraying with the biopesticide (onion extracts) was done using a hand

sprayer early in the morning, as recommended by Vijayalakshmi *et al.* (1999) at 7-day intervals. The controls were sprayed at the same time with distilled water.

3.6 Data collection

3.6.1 Plant growth parameter

The emergence date of every seedling was recorded independently, and used to determine the duration for germination. The number of plants that emerged per row was counted to determine the percentage germination. Date for formation of the first three leaves was recorded. When the first 3 leaves had been fully formed in about 80% of the plants, plant heights were measured by a tape measure and recorded in centimeters. This was repeated on weekly basis to determine the rate of plant growth. Plants with deformed leaves were recorded.

3.6.2 Arthropod populations

Screw-capped containers each containing 10 ml of 70% ethanol were placed on every treatment row of 20 plants. Aphids and other arthropods from every plants per row were collected into each container using a camel hair brush from leaves and stems. The collected arthropods were identified and counted in the laboratory at $\times 10$ magnifications using a dissecting microscope (Leica ZOOM 2000, Model Z45E, Leica Inc., Buffalo, NY U.S.A.)

3.7 Statistical analysis

Statistical analyses were conducted using SAS 9.1 software (SAS Institute Inc) at $p \leq 0.05$ confidence level. Descriptive statistics such as means and standard errors for leaf deformation, plant height and biomass parameters were generated using proc means. Data

on plant growth were checked for normality using proc univariate. Proc glm was used for the analyses of variance (ANOVA) among the treatments and means were separated using student's t-test in ls means when the ANOVA was significant. Data on aphid and mite populations were analyzed using proc genmod (Poisson) and means separated using proc multtest

CHAPTER FOUR

RESULTS

4.1 Mean arthropod populations on plants

At least six morphologically distinct arthropods were identified in the experimental fields. These arthropods included the black bean aphid *Aphis fabae* (black bean aphids) whose color and shape of the cauda fitted the description by Blackman and Eastop (2006) (Plate 4.1). Mites were identified as *Tetranychus evansi* (red spider mites) were identified based on body size, shape and tarsal setae (Ben-David *et al.*, 2007) (Plate 4.2). Ants (Formicidae) sampled and using keys of Lim and Pickering (2008) included *Dolichoderus mariae* (Plate 4.3a, 4.3b), *Formica glarialis* (Plate 4.4), *F. padelifulva* (Plate 4.5) and *Camponotus* (Plate 4.6); with *D. mariae* being the dominant species.



Plate 4.1. Apterous *Aphis fabae* Linnaeus



Plate 4.2. *Tetranychus evansi* Linnaeus

(Source: Author, 2012)



Plate 4.3a. *Dolichoderus mariae* Linnaeus



Plate 4.3b *Dolichoderus mariae* Linnaeus



Plate 4.4. *Formica glarialis* Linnaeus



Plate 4.5 *Formica padellifulva*

Linnaeus



Plate 4.6. *Camponotus* Linnaeus (Source: Author, 2012)

4.2 Aphid (*Aphis fabae*) population and interactions with cultivars and manure

In trial 1, interactions between cultivar and manure treatments significantly affected aphid populations ($df=1$, $\chi^2=1.10$; $p<0.05$) (Table 4.1) *S. nigrum var. nigrum* cultivar B grown with chicken manure had the highest aphid population; *S. nigrum var. scabrum*, cultivar M grown with manure C or without fertilizer had intermediate aphid population; but the number of aphids was low in the remaining six treatment combinations (Table 4.1)

In trial 2, there were significant interactions between cultivars, manure types and the sprays on aphid populations. ($df=17$; $\chi^2=374.96$; $p < 0.05$) (Table 4.2). Among the controls (water spray), cultivars B (*S. nigrum var. nigrum*) and M (*S. nigrum var. scabrum* from market) had the highest aphid populations when fertilized with chicken manure. This was followed by those without manure application while plants fertilized with cattle manure had the least aphid populations. The aphid populations in cultivar F (*S. nigrum var. scabrum* from University Farm) were statistically not different for plants receiving chicken manure and those without manure.

Table 4.1. Effect of manure on aphid (*A. fabae*) populations infesting *S. nigrum* cultivars

Source of variation	Treatment	Number of Aphids per plant
Cultivar B	Chicken manure	11.5±4.20 a
Cultivar M	Cattle manure	6.2±1.21 b
Cultivar M	No manure	5.8±1.18 bc
Cultivar F	Chicken manure	4.5±1.20 cd
Cultivar M	Chicken manure	4.4±1.19 d
Cultivar F	Cattle manure	4.4±1.19 d
Cultivar B	No manure	4±1.19 d
Cultivar F	No manure	4±1.19 d
Cultivar B	Cattle manure	3.6±0.79 d

Source of variation	df	Chi- Square
Cultivar x Manure	8	32.27***

Treatment means followed by the same letter within a particular column are statistically not different. *Asterisk indicate the significance level *** $P \leq 0.001$ ** $P \leq 0.01$ * $P \leq 0.05$

These aphid populations were significantly higher than those treated with cattle manure. Among plants sprayed with onion extracts, cultivar B (*S. nigrum* var. *nigrum*) had the highest aphid population when grown without manure. This was followed by those grown with chicken manure while plants treated with cattle manure had the lowest aphid populations. For cultivar F (*S. nigrum* var. *scabrum* from University Farm), the highest aphid numbers were attained when grown with chicken manure, followed by those without manure.

Plants treated with cattle manure had the lowest aphid populations. In cultivar M (*S. nigrum* var. *scabrum* from market), plants treated with chicken manure and those without manure were statistically not different. These had significantly higher aphid populations than those treated with cattle manure. Within cultivar B (*S. nigrum* var. *nigrum*), the aphid populations were highest when the plants were sprayed with water and grown without manure, though statistically not different when the plants were treated with chicken manure and sprayed with onion extracts. When the plants were treated with cattle manure, aphid populations were lowest when onion extracts were used. Within cultivar F (*S. nigrum* var. *scabrum* from University Farm) across all the manure types, aphid populations were higher among the controls than among those sprayed with onion extracts. Within cultivar M (*S. nigrum* var. *scabrum* from market), the aphid populations were not statistically different among the plants with no manure treatment, in both the control and onion extract sprayed plants. When grown with chicken manure, the plants had higher aphid populations when sprayed with onion extracts than among the controls. In the same cultivar (M), there were higher aphid populations in the controls than in those sprayed with onion extracts when the plants were grown with cattle manure. Among

plants that had received no manure treatments, aphid populations in B (*S. nigrum var. nigrum*) the highest among the controls, followed by F among the controls. Aphid populations among the controls were lowest in the three cultivars, but not statistically different from the three cultivars when sprayed with the onion extracts. In plants that had been treated with cattle manure, there was no statistical difference in aphid populations between cultivars B (*S. nigrum var. nigrum*) and M (*S. nigrum var. scabrum* from market) when sprayed with onion extracts. The aphid populations were statistically higher than in cultivar F (*S. nigrum var. scabrum* from university farm).

When treated with cattle manure and sprayed with the water, the three cultivars were statistically different in aphid populations. Cultivar B (*S. nigrum var. nigrum*) had the highest aphid populations, followed by F (*S. nigrum var. scabrum* from University Farm). Cultivar M (*Solanum nigrum var. scabrum*) had the lowest aphid populations. Among plants treated with chicken manure, the three cultivars had statistical differences in aphid populations when sprayed with onion extracts. Statistically, cultivar B (*S. nigrum var. nigrum*) had the highest aphid populations, followed by cultivar M (*S. nigrum var. scabrum* from market). Cultivar F (*S. nigrum var. scabrum* from University Farm) had statistically the lowest aphid populations.

In plants treated with chicken manure and sprayed with water, there were statistical differences in aphid populations. Cultivar F (*S. nigrum var. scabrum* from University Farm) had the highest aphid populations, followed by B (*S. nigrum var. nigrum*).

Cultivar M (*S. nigrum var. scabrum* from market) had the lowest aphid populations. Considering individual Cultivars, B (*S. nigrum var. nigrum*) when sprayed with onion

extracts had the highest aphid populations in the plants which had been treated with chicken manure, followed by the plants which had no manure treatments. The treated plants had the lowest aphid populations

Table 4.2 Mean number of aphids (*Aphis fabae*) collected per plant

Source of variation	Cultivar	Treatment	Means of number <i>Aphis fabae</i>
Control	B	No manure	8.5±1.97 def
	F	No manure	9.2±1.98 d
	M	No manure	7.8±1.64 ef
	B	Cattle manure	4.6±1.19 g
	F	Cattle manure	2.4±1.10 h
	M	Cattle manure	4.6±1.19 g
	B	Chicken manure	16.2±4.44 a
	F	Chicken manure	9±2.58 de
	M	Chicken manure	10.7±4.27 c
Sprayed	F	No manure	11.2±3.93 c
	M	No manure	7.6±1.33 f
	B	Cattle manure	7.2±1.33 f
	F	Cattle manure	5.5±1.25 g
	M	Cattle manure	3.3±0.76 g
	B	Chicken manure	11.7±4.22 c
	F	Chicken manure	14.1±3.25 b
	M	Chicken manure	7.7±2.01 f
	Source of variation		df
Cultivar x manure x spray		17	1019.52***

Treatment means followed by the same letter within a particular column are statistically not different. $P \leq 0.05$.

When sprayed with the onion extract, the plants had the highest aphid populations in the plants which had no manure treatments. This was followed by the plants which had chicken manure treatments. Plants treated with cattle manure had the lowest aphid populations. In cultivar F (*S. nigrum var. scabrum* from University Farm), the plants which had no manure treatments had the highest aphid populations, though statistically not different from those treated with chicken manure, when sprayed with onion extracts. The plants treated with cattle manure had the lowest aphid populations. When sprayed with water, cultivar F (*S. nigrum var. scabrum* from University Farm) had the highest aphid populations. Plants treated with chicken manure were followed by the plants which had no manure treatments. Plants treated with cattle manure had the lowest aphid populations. Cultivar M (*S. nigrum var. scabrum* from market) which had been treated with chicken manure when sprayed with onion extracts, followed by the plants which had no manure treatments. Plants treated with cattle manure had the lowest aphid populations. In plants sprayed with water, there was no statistical difference between plants which had been treated with chicken manure and those that had no manure treatments. Plants treated with cattle manure had the lowest aphid populations.

4.3 Mite populations and interaction with cultivars and manure

In trial 1, mite populations were not statistically different in all the three manure types ($p > 0.05$). In trial 2, there were significant differences in mite populations between the cultivars, manure types and onion extract sprays ($df=17$; $\chi^2=1019.52$; $p<0.05$) (Table 4.3). Among the controls, cultivar B (*S. nigrum var. nigrum*) and M had the highest mite populations of 10 when grown without manure. In this cultivar, the mite populations were lowest in plants growth with chicken manure and in those with cattle manure. In cultivar

F (*S. nigrum* var. *scabrum* from university farm), the mite populations were highest in plants grown with cattle manure. These were followed by those grown without manure, while plants grown with chicken manure had the lowest mite populations. Among plants sprayed with onion extracts, cultivar B (*S. nigrum* var. *nigrum*) had the highest mite populations when grown without manure. This was followed by cultivar F (*S. nigrum* var. *scabrum* from university farm) when grown with chicken manure, though there was no statistical difference with the same cultivar when grown without manure. This was followed by cultivar B (*S. nigrum* var. *nigrum*) when grown with chicken manure in terms of pest populations. Cultivar M (*S. nigrum* var. *scabrum* from market) when grown without manure and F (*S. nigrum* var. *scabrum* from University Farm) grown with cattle manure had the highest mite populations, though the mite populations were not statistically different from the populations on M (*S. nigrum* var. *scabrum* from market) when grown with chicken manure. Cultivar B (*S. nigrum* var. *nigrum*) had the lowest mite populations when grown with cattle manure.

Table 4.3 Mean number of mites collected per plant

Source of variation	Cultivar	Treatment	Means of <i>Tetranychus evansi</i>
Control	B	No manure	10.7 ±2.99 cde
	F	No manure	10.6±2.99 def
	M	No manure	16.6±3.56 a
	B	Cattle manure	8.9±2.03 fgh
	F	Cattle manure	12.2±2.25 bc
	M	Cattle manure	11.6±3.03 cd
	B	Chicken manure	8.7±2.07 gh
	F	Chicken manure	9±2.01 fg
	M	Chicken manure	11.3±2.15 cd
Sprayed	B	No manure	13.9±3.85 b
	F	No manure	11±3.00 cd
	M	No manure	7.4±1.76 hi
	B	Cattle manure	4±0.96 j
	F	Cattle manure	7.4±1.76 hi
	M	Cattle manure	11.9±2.24 cd
	B	Chicken manure	9.1±2.01 efg
	F	Chicken manure	11.9±2.24 cd
	M	Chicken manure	6.8±1.23 i
Source of variation		df	<i>Tetranychus evansi</i>
Cultivar x manure x spray		17	374.96***

Treatment means followed by the same letter within a particular column are statistically not different. $P \leq 0.05$.

This was followed by plants which had been treated with cattle manure, though not statistically different from the populations on plants that had been treated with chicken manure. Among plants that were sprayed with onion extracts, the plants with no manure treatment had the highest mite populations, followed by plants that had received chicken manure treatments. Plants which had been treated with chicken manure had the lowest mite populations. When sprayed with water, cultivar F (*S. nigrum* var. *scabrum* from university farm) had the highest mite populations in plants which had been treated with cattle manure, followed with those with no manure treatment, though statistically not different from the populations on the plants that had been treated with cattle manure.

When sprayed with onion extracts, the mite populations on plants with no manure treatment were not statistically different from the populations on plants treated with chicken manure. Plants that received cattle manure treatment had lower mite populations. Considering cultivar M (*S. nigrum* var. *scabrum* from market), when sprayed with water, mite populations were highest on plants that had no manure treatment. Plants that had received chicken and cattle manure respectively had lower mite populations, which were statistically not different. When sprayed with onion extracts, the plants that had been treated with cattle manure had the highest mite populations followed by those that had received no manure treatments. Plants with the lowest mite populations were those that had received chicken manure treatment, though not statistically different from the populations on the plants with no manure treatment.

4.4 Mean ant populations on plants

In trial 1, ant populations were not different. In trial 2, there were significant differences in ant populations between plant cultivars, manure types and onion extract sprays (df=17; Chi-value = 92.25; $p < 0.05$) (Table 4.4).

Among the plants that had received no manure treatment and sprayed with water, cultivars B (*S. nigrum* var. *nigrum*) and M (*S. nigrum* var. *scabrum* from market) had no statistical difference in ant populations. These plants had higher ant populations than cultivar F (*S. nigrum* var. *scabrum* from university farm), though statistically F (*S. nigrum* var. *scabrum* from University Farm) was not different from B (*S. nigrum* var. *nigrum*) and M in ant populations. In the plants treated with cattle manure, ant populations were higher on cultivars B (*S. nigrum* var. *nigrum*) and M (*S. nigrum* var. *scabrum* from market), though not statistically different from the populations on the cultivar F (*S. nigrum* var. *scabrum* from University Farm). In plants treated with chicken manure, the ant populations were highest on cultivar M (*S. nigrum* var. *scabrum* from market), followed by B (*S. nigrum* var. *nigrum*), though the ant populations on the three cultivars were statistically not different.

Among the plants that had received no manure treatments, and sprayed with onion extracts, cultivar B (*S. nigrum* var. *nigrum*) had the highest ant populations, followed by cultivar F (*S. nigrum* var. *scabrum* from University Farm). The cultivar M (*S. nigrum* var. *scabrum* from market) had the lowest ant populations, though not statistically different from the populations on cultivar F (*S. nigrum* var. *scabrum* from University Farm). In

plants which were treated with cattle manure and sprayed with the onion extract, cultivar M (*S. nigrum* var. *scabrum* from market) had the highest ant population

Table 4.4 Mean ant populations per plant

Source of variation	Cultivar	Treatment	Means of ants
Control	B	No manure	0.5 ±0.20 bcd
	F	No manure	0.3 ±0.33 cd
	M	No manure	0.5 ±0.19 bcd
	B	Cattle manure	0.5 ±0.29 bcd
	F	Cattle manure	0.3 ±0.33 cd
	M	Cattle manure	0.5 ±0.19 bcd
	B	Chicken manure	0.5 ±0.29 bcd
	F	Chicken manure	0.5 ±0.21 bcd
	M	Chicken manure	0.6 ±0.21 bc
Sprayed	B	No manure	0.7 ±0.38 b
	F	No manure	0.4 ±0.13 bcd
	M	No manure	0.3 ±0.46 d
	B	Cattle manure	0.3 ±0.10 cd
	F	Cattle manure	0.3 ±0.10 cd
	M	Cattle manure	0.6 ±0.25 bc
	B	Chicken manure	0.4±0.13 bcd
	F	Chicken manure	1.8 ±0.5 a
	M	Chicken manure	0.3 ±0.15 cd
Source of variation		df	Chi-square
Cultivar x manure x spray		17	92.25***

Treatment means followed by the same letter within a particular column are statistically not different. $P \leq 0.05$.

Cultivars B (*S. nigrum* var. *nigrum*) and F had lower ant populations and statistically not different from one another. In plants treated with chicken manure and sprayed with the onion extract cultivar F (*S. nigrum* var. *scabrum* from University Farm) had the highest ant population, followed by B (*S. nigrum* var. *nigrum*). Cultivar M had the lowest ant populations, though statistically not different from B (*S. nigrum* var. *nigrum*). Considering individual plant cultivars, in cultivar B (*S. nigrum* var. *nigrum*), ant populations were statistically not different on plants which had no manure treatment or treated with manure when sprayed with water. When onion extract was applied, the plants with no manure treatment had the highest ant populations, followed by those plants treated with chicken manure. Plants treated with cattle manure had the lowest ant populations, though statistically not different from those that were treated with chicken manure. In cultivar F (*S. nigrum* var. *scabrum* from University Farm), when sprayed with water, plants treated with chicken manure had the highest ant populations, though not statistically different from the populations on those plants treated with cattle manure and those that received no manure treatment. Among those plants that were sprayed with onion extracts, the plants that received chicken manure treatment had the highest ant populations. They were followed by the populations on those plants that had received no manure treatments. Plants that had been treated with cattle manure had the lowest ant populations, though not statistically different from the populations on the plants that had received no manure treatments.

4.5 Correlations between arthropod populations

There was a positive correlation between aphid and mite populations ($p = 0.05$). As aphid populations increased, the population the mite populations also increased. There was also

a positive correlation between aphids and ants ($p < 0.05$). The ant populations increased with increasing aphid populations. On the other hand, there was no correlation between ant and mite populations.

4.6 Mean heights of plants

In trial 1, there were significant interactions between cultivar and manure treatments on plant height ($P=0.05$) (Table 4.5). In cultivar M (*S. nigrum* var. *scabrum* from market), plants treated with cattle manure were taller than those plants grown with chicken manure and the ones without manure, the last two not being statistically different. In cultivars B (*S. nigrum* var. *nigrum*) and F (*S. nigrum* var. *scabrum* from University Farm), plant height was not different between those grown with cattle manure, chicken manure and the controls. Among the controls and those grown with cattle manure, plants of cultivar F were taller than those of cultivar B (*S. nigrum* var. *nigrum*), while cultivar M (*S. nigrum* var. *scabrum* from market) was intermediate and not statistically different from the previous two. In plants grown with chicken manure, those of cultivar M (*S. nigrum* var. *scabrum* from market) were similar in height to cultivar F (*S. nigrum* var. *scabrum* from University Farm), but both were taller than plants of cultivar B (*S. nigrum* var. *nigrum*).

In trial 2, there were significant differences in plant height between the three cultivars ($p < 0.05$) (Table 4.5). Cultivar B (*S. nigrum* var. *nigrum*) was significantly shorter than M (*S. nigrum* var. *scabrum* from market) and F (*S. nigrum* var. *scabrum* from University Farm) which were statistically not different. In the same trial, plants treated with cattle manure and chicken manure were significantly taller than those that did not receive any manure ($p = 0.05$) (Table 4.5).

Table 4.5 Means of plant heights in centimeters

Source of variation	Manure treatments	First trial	Second trial
		Mean plant heights in centimeters	
Cultivars	B	18.0±1.3	21.5±1.0 b
	F	28.7±1.3	28.1±1.1 a
	M	26.9±1.4	28.6±1.0 a
Manure	Cattle manure	25.0±1.3	27.0±1.0 a
	Chicken manure	27.7±1.5	28.9±1.1 a
	None	22.6±1.3	22.9±1.0 b
Cultivar B	Cattle manure	21.0±2.5 cd	20.8±1.8
	Chicken manure	14.4±1.4 e	23.6±1.7
	None	18.5±2.3 d	19.9±1.8
Cultivar F	Cattle manure	27.5±2.1 abc	27.7±1.7
	Chicken manure	31.9±2.5 ab	31.4±1.9
	None	26.6±2.3 bc	25.1±1.8
Cultivar M	Cattle manure	25.5±2.1 c	31.3±1.7
	Chicken manure	33.7±2.6 a	30.9±1.7
	None	21.6±2.0 cd	23.5±1.6
Source of variation	Df	F	Values
Cultivar	2	17.13***	15.25***
Manure	2	2.77	8.18***
Cultivar × Manure	4	3.75**	1.16

Treatment means followed by the same letter within a particular column are statistically not different. *Asterisk indicate the significance level *** $P \leq 0.001$ ** $P \leq 0.01$ * $P \leq 0.05$

4.7 Mean number of deformed leaves per plant

There were significant differences in leaf deformation between the two trials ($df=1$; $\chi^2=21.4$; $p < 0.05$)(Table 4.6). There were significant differences in leaf deformation between the cultivars ($df=2$; $\chi^2=36.94$; $p < 0.05$). Variety (*S. nigrum var. nigrum*) B had fewer deformed leaves when compared to varieties F and M (*S. nigrum var. scabrum* from market), which were not statistically different. There was significant difference in leaf deformation between the manures and spray treatments ($df=2$; $\chi^2=15.35$; $p=0.05$) (Table 4:3).

Among plants sprayed with water, there was no statistical difference between plants treated with chicken manure and those without manure. These had fewer deformed leaves when compared to those treated with cattle manure. In plants sprayed with onion extracts, plants treated with cattle manure and those without manure were statistically not different. The number of deformed leaves in these manures was significantly lower than in chicken manure.

Table 4.6 : Mean number of deformed leaves per plant.

Source of variation	Treatments	Mean deformed leaves per plant
Trial	1	1.1±0.08 b
	2	1.5±0.07 a
Cultivar	B	1±0.08 b
	F	1.4±0.09 a
	M	1.6±0.09 a
Controls	Cattle manure	1.4±0.11 a
	Chicken manure	1.2±0.10 b
	None	1.3±0.11 b
Sprayed	Cattle manure	1.3±0.14 b
	Chicken manure	1.8±0.19 a
	None	1.3±0.14 b

Source of variation	Df	Chi-VALUE
Trial	1	21.4***
Cultivar	2	36.94***
Manure × Spray	2	15.35***

Treatment means followed by the same letter within a particular column are statistically not different. *Asterisk indicate the significance level, *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$

4.8 Plant Biomass

4.8.1. Mean plant fresh weight

There were significant differences in fresh weights between the cultivars, manure and onion extract sprays ($df = 4$; $F = 3.02$; $p = 0.05$).

Controls of cultivar M (*S. nigrum* var. *scabrum* from market) had the highest fresh weight when grown with cattle manure; although this weight was statistically not different from that of plants sprayed with onion extract in the same manure and cultivar. These were followed by the controls of cultivar F (*S. nigrum* var. *scabrum* from University Farm) and plants of cultivars F (*S. nigrum* var. *scabrum* from University Farm) and M (*S. nigrum* var. *scabrum* from market) sprayed with onion extract all grown chicken manure; although their fresh weights were not different from cultivar M (*S. nigrum* var. *scabrum* from market) sprayed with onion extract and fertilized with cattle manure. These were followed by the controls of cultivar M (*S. nigrum* var. *scabrum* from market) and plants of cultivar B (*S. nigrum* var. *nigrum*) sprayed with onion extract plants both grown with chicken manure; and controls of cultivar F (*S. nigrum* var. *scabrum* from University Farm) grown with cattle manure. These were followed by cultivar M (*S. nigrum* var. *scabrum* from market) grown without manure and cultivar F (*S. nigrum* var. *scabrum* from University Farm) grown with either cattle manure or without manure all sprayed with onion extracts; and the controls of cultivars F (*S. nigrum* var. *scabrum* from university farm) and B (*S. nigrum* var. *nigrum*) both grown without manure.

The lowest fresh weights were attained by cultivar B (*S. nigrum* var. *nigrum*) grown with either cattle manure or without manure both sprayed with onion extract, the controls of cultivar M (*S. nigrum* var. *scabrum* from market) grown without manure; and the controls of cultivar B (*S. nigrum* var. *nigrum*) grown with either chicken manure or cattle manure.

Among plants without manure, controls of cultivar M (*S. nigrum* var. *scabrum* from market) and plants of cultivar B (*S. nigrum* var. *nigrum*) sprayed with onion extract were statistically not different. These had the lowest fresh weight, although not different from plants of cultivar F (*S. nigrum* var. *scabrum* from University Farm) sprayed with onion extract. Controls of cultivars B (*S. nigrum* var. *nigrum*) and F (*S. nigrum* var. *scabrum* from university farm) and plants of cultivars F (*S. nigrum* var. *scabrum* from University Farm) and M (*S. nigrum* var. *scabrum* from market) both sprayed with onion extract were statistically not different with the highest fresh weight.

Table 4.7 : Mean dry weights per plant in grams

Source of variation	Cultivars	Mean dry weight of plant in grams
Controls sprayed with water	B	97.2±7.4 h
	B	108.9±9.7 fgh
	B	144.5±16.5 cdef
	F	144.5±9.1 cdef
	F	172.1±12.2 bc
	F	132.1±11.6 defg
	M	207.3±15.3 a
	M	144.4±13.3 cdef
	M	102.8±9.2 gh
Sprayed with onion extract	B	118.1±11.7 efgh
	B	159.0±11.2 cde
	B	105.0±10.6 fgh
	F	159.2±12.4 cd
	F	172.5±9.9 bc
	F	153.8±13.4 cde
	M	192.8±14.1 ab
	M	158.3±10.5 cde
	M	144.3±12.5 cdef
Source of variation	Df	F values
Cultivar	2	12.12***
Manure	2	6**
Spray	1	3.99*
Cultivar × manure	4	8.97***
Cultivar × spray	2	0.02
Manure × spray	2	0.61
Spray × Cultivar × Manure	4	3.69**

Treatment means followed by the same letter within a particular column are statistically not different. *Asterisk indicate the significance level, *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$

Among the plants treated with cattle manure, cultivar M (*S. nigrum* var. *scabrum* from market) had the highest fresh weight when sprayed with either water or onion extract. These were followed by cultivar F (*S. nigrum* var. *scabrum* from University Farm) sprayed with either water or onion extract. Cultivar B (*S. nigrum* var. *nigrum*) had the lowest fresh weight when sprayed with either water or onion extract; although the fresh weight of cultivars B (*S. nigrum* var. *nigrum*) and F were statistically not different when sprayed with onion extract.

Among the controls, cultivar M (*S. nigrum* var. *scabrum* from market) grown with cattle manure had the highest fresh weight, then cultivar F grown with chicken manure. These were followed by cultivar B (*Solanum nigrum* var. *nigrum*) grown without fertilizer, Cultivar M (*S. nigrum* var. *scabrum* from market) grown with chicken manure and cultivar F (*S. nigrum* var. *scabrum* from University Farm) grown with cattle manure which were statistically not different. However, the fresh weight of cultivar M (*S. nigrum* var. *scabrum* from market) grown with chicken manure and cultivar F (*S. nigrum* var. *scabrum* from university farm) grown with cattle manure not different from that of cultivar F (*S. nigrum* var. *scabrum* from University Farm) grown with chicken manure.

Cultivars F and M (*S. nigrum* var. *scabrum* from market) grown without manure and cultivar B (*S. nigrum* var. *nigrum*) grown with either chicken manure or cattle manure had the lowest fresh weight although the weights of cultivar B (*S. nigrum* var. *nigrum*) grown with chicken manure and those of cultivar F grown without manure were not different from those of cultivar B (*S. nigrum* var. *nigrum*) grown without manure.

Among plants sprayed with onion extract, cultivar F (*S. nigrum* var. *scabrum* from university farm) grown with chicken manure and cultivar M (*S. nigrum* var. *scabrum* from market) grown with either chicken manure or cattle manure had the highest fresh weight. These were followed by cultivars M (*S. nigrum* var. *scabrum* from market) and F (*S. nigrum* var. *scabrum* from university farm) grown without manure, cultivar B (*S. nigrum* var. *nigrum*) grown with chicken manure and cultivar F grown with cattle manure, although these were not different from cultivar M grown with chicken manure. Cultivar B (*S. nigrum* var. *nigrum*) grown with either cattle manure or without manure had the lowest fresh weight.

However, this weight was not different from that of cultivars M (*S. nigrum* var. *scabrum* from market) and F (*S. nigrum* var. *scabrum* from University Farm) grown without manure, cultivar B (*S. nigrum* var. *nigrum*) grown with chicken manure and cultivar F (*S. nigrum* var. *scabrum* from University Farm) grown with cattle manure. Among the plants in cultivar B (*S. nigrum* var. *nigrum*) when sprayed with the onion extract, the plants without manure treatment had the lowest fresh weight, followed by the plants which had received cattle manure treatment though there was no statistical difference between the fresh weights of the two. The plants that had received chicken manure treatment had the highest fresh weight.

Cultivar F (*S. nigrum* var. *scabrum* from University Farm), when sprayed with water, the plants that were without manure treatments had the lowest fresh weight. These were followed by the plants which had received cattle manure treatment, though the two were not statistically different. Plants with chicken manure treatment had the highest fresh

weight. When sprayed with the onion extracts, the fresh weight of the plants that had cattle manure treatment and those without manure treatment had no statistical difference.

The plants that had received chicken manure treatment had the highest fresh weight. In cultivar M (*S. nigrum* var. *scabrum* from market), when the plants were sprayed with water, those without manure treatments had the lowest fresh weight, these were followed by the plants that had chicken manure treatment. The plants that had received cattle manure treatments had the highest fresh weight. Among the plants that were sprayed with onion extracts, the plants that were without manure treatment had the lowest fresh weight. These were followed by the plants that had received chicken manure treatment, though there was no statistical difference in weight between the two treatments. The plants that had received cattle manure treatment had the highest fresh weight, though there was no statistical difference in fresh weight between the plants that had received cattle manure treatment and those that had been treated with chicken manure.

4.8.2 Mean plant dry weight

There were significant differences in dry weights between the cultivars, manure and onion extract sprays ($df = 4$; $F = 3.69$; $P = 0.05$) (Table 4.7).

Among plants without manure treatment, and sprayed with water, cultivar B (*S. nigrum* var. *nigrum*) had the highest dry weight, followed by cultivar F (*S. nigrum* var. *scabrum* from university farm), though there was no statistical difference between dry weights in cultivars B (*S. nigrum* var. *nigrum*) and F (*S. nigrum* var. *scabrum* from university farm). Cultivar M (*S. nigrum* var. *scabrum* from market) had the lowest fresh weight, though not statistically different from that of cultivar F (*S. nigrum* var. *scabrum* from university

farm). In the plants which had received chicken manure treatment and sprayed with water, cultivar B (*S. nigrum* var. *nigrum*) had the lowest dry weight, followed by cultivar M (*S. nigrum* var. *scabrum* from market). Cultivar F (*S. nigrum* var. *scabrum* from University Farm) had the highest dry weight, though not statistically different from cultivar M (*S. nigrum* var. *scabrum* from market). Among the plants that had received cattle manure treatment and sprayed with water, cultivar B (*S. nigrum* var. *nigrum*) had the lowest dry weight, followed by cultivar F (*S. nigrum* var. *scabrum* from university farm) plants. Cultivar M (*S. nigrum* var. *scabrum* from market) had the highest plant dry weight. Among the plants that were sprayed with onion extracts, cultivar B (*S. nigrum* var. *nigrum*) had the lowest dry weight among the plants that were without manure treatment. Cultivars F (*S. nigrum* var. *scabrum* from university farm) and M (*S. nigrum* var. *scabrum* from market) had no statistical difference in fresh dry.

Cultivar B (*S. nigrum* var. *nigrum*) had the lowest dry weight among the plants treated with chicken manure, followed by cultivar M (*S. nigrum* var. *scabrum* from market), cultivar F (*S. nigrum* var. *scabrum* from University Farm) having the highest dry weight, though the three cultivars had no statistical difference in dry weight. Among the plants treated with cattle manure, cultivar B (*S. nigrum* var. *nigrum*) had the lowest dry weight followed by cultivar F (*S. nigrum* var. *scabrum* from University Farm), though the two were not statistically different. Cultivar M (*S. nigrum* var. *scabrum* from market) had the highest dry weight.

When individual plant cultivars are considered, B (*S. nigrum* var. *nigrum*) when sprayed with water had the highest dry weight when the plants were without manure treatment, followed by plants which had been treated with chicken manure, though the two were

statistically not different. The plants that had cattle manure treatment had the lowest dry weight, though not statistically different from the plants that had chicken manure treatment. Among the plants in cultivar B (*S. nigrum* var. *nigrum*) when sprayed with the onion extract, the plants without manure treatment had the lowest dry weight, followed by the plants which had received cattle manure treatment though there was no statistical difference between the fresh weights of the two. The plants that had received chicken manure treatment had the highest dry weight.

Cultivar F (*S. nigrum* var. *scabrum* from University Farm), when sprayed with water, the plants that without manure treatments had the lowest dry weight. These were followed by the plants which had received cattle manure treatment, though the two were not statistically different. Plants with chicken manure treatment had the highest dry weight. When sprayed with the onion extracts, the dry weight of the plants that had cattle manure treatment and those without manure treatment had no statistical difference. The plants that had received chicken manure treatment had the highest dry weight.

In cultivar M (*S. nigrum* var. *scabrum* from market), when the plants were sprayed with water, those without manure treatments had the lowest dry weight, these were followed by the plants that had chicken manure treatment. The plants that had received cattle manure treatments had the highest dry weight. Among the plants that were sprayed with onion extracts, the plants that were without manure treatment had the lowest dry weight. These were followed by the plants that had received chicken manure treatment, though there was no statistical difference in weight between the two treatments. The plants that had received cattle manure treatment had the highest dry weight, though there was no statistical difference in fresh weight between the plants that had received cattle manure

treatment and those that had been treated with chicken manure. Similar trends were taken by all the three cultivars in terms of dry weight.

CHAPTER FIVE

DISCUSSION

5.1. Infestations by aphids, mites and ants

Nightshades (*S. nigrum*) exhibit high demands in western Kenya due to their high nutritional and medicinal values. Currently, arthropod pests such as the black bean aphid and the red spider mite have become a major problem in western Kenya, especially for improved cultivars that are being adopted in this region. The present research integrated the use of different plant cultivars of *S. nigrum* complex, aqueous *A. cepa* extracts and organic manure in the control of *A. fabae* and *T. evansi* infesting *S. nigrum* cultivars under field conditions in western Kenya. It had been hypothesized that *S. nigrum* cultivars vary in their biological attributes that make them have different levels of susceptibility to *A. fabae* and *T. evansi*, while combined application of *A. cepa* extracts and farmyard manures could enhance protection of the vegetable crop against the pests.

It was expected that pest populations will vary between cultivars, and the application of farmyard manures and *A. cepa* extracts would reduce these infestations. Aphids and their symbiont populations varied between nightshades cultivars. *Solanum nigrum* var. *nigrum* (Cultivar B) had the highest population of *A. fabae* and their attendant ant populations when planted without manure treatments. *Solanum nigrum* var. *scabrum* (Cultivar M) from the open market had the lowest *A. fabae* and symbiotic ant populations. On the other hand, *S. nigrum* var. *scabrum* (Cultivar B) had lower number of deformed leaves than Cultivar F and Cultivar M. The findings indicate that cultivar B is more tolerant to aphids since it expressed low leaf deformations despite having higher aphid populations than Cultivars F and M. This confirms the

expectation that the three cultivars would vary in pest infestations and their associated damages.

Combined application of cattle manure and onion extract spray reduced *A. fabae* population in *S. nigrum* var. *scabrum* Cultivar M and Cultivar F. This implies that Cultivars M and F readily respond to the pest control by onion extract spray, especially when organic amendments like cattle manure are used. It is likely that plant extracts used as botanical pesticides work best depending on the type of manure used. Botanical plant extracts such as neem *Azadirachta indica* (Meliaceae) and *Melia azadrachta* have been found to control aphids, cowpea weevil and termites in cowpeas and brassicas (Elwell and Maas, 1995; Shiberu *et al.*, 2014). Onions contain allinin and sulphur compounds that are linked to the defense of plants against pests (Hell, 1997; Crawford *et al.*, 2000; Leustek *et al.*, 2000; Saito, 2004). These could have shown their effects although the refined contents of onion were not used in the present study.

Aphis fabae and their symbiotic ant populations appeared to increase in Cultivars F and B when the onion extract was sprayed on plants fertilized with chicken manure.

The reason for this drastic increase of the pest populations on the plants of cultivar F when treated with chicken manure and sprayed with onion extracts could not be established; contradicts the hypothesis. The present study found that cultivars vary in their susceptibility to aphids and mites. This was also reflected in leaf damage by the arthropods and plant biomass and height respectively. *Solanum nigrum* var. *nigrum*. Cultivar B had the lowest number of deformed leaves. Although cultivar B had the highest aphid, symbiotic ant and mite populations, the cultivar had the lowest number of deformed leaves; this indicates that cultivar B is tolerant to aphid and mite infestations. Cultivar B also showed a significant difference in terms of height and

biomass when compared to other cultivars F and M. This suggests that cultivar B is tolerant to the pests but less productive. Sanford *et al.* (1992) reported that *S. nigrum* cultivars vary in resistance to pests and pathogens

5.2. Seasonal Variations of Pest Populations

Aphid populations and their symbionts were low in the first trial (long rain season) compared to the second trial (short rain season). It is possible that the heavy rains experienced during the first trial could have reduced the reproductive success and survival of *A. fabae* on the three selected *S. nigrum* cultivars. The high emergence of *A. fabae* during the second trial might have been as a result of the carry over population of these pests from the first trial and also the relatively low rains which were experienced during this season could have favored high reproductive potential and survival for the aphids and thus attracting an increase in their attendant ants. These results are similar to those reported by Hasan *et al.* (2009) where the aphid populations on Mustard variety “Sampad” declined due to the influence of rainfall.

Climatic factors such as temperature and humidity also affect aphid populations (Hasan *et al.*, 2009). The population of the red spider mite *T. evansi* did not attain significant levels in the first season. The insignificant levels of red spider mites (*T. evansi*) during the first trial might have resulted from the prevailing climatic conditions. The second trial experienced high mite populations on the three nightshade cultivars. In this study there was a variation in infestation levels of *T. evansi*, across the different cultivars of *S. nigrum* during the second (short rain) season. The results suggest that some pests, such as red spider mites, take a longer time to build up a significant population and infestation of 20 mites per plant. This finding seems to agree with the findings of Steinkraus *et al.*, (1999) on the spider mites population reduction on cotton on the increase in the amount of rain.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Based on the present study, it is concluded that *S. nigrum* var. *nigrum* Cultivar B is more tolerant to *A. fabae* and *T. evansi* infestations than *S. nigrum* var. *scabrum* cultivars F and M.

A combination of cattle manure and onions (*A. cepa*) extract spray may have considerable effects on the aphid and mite populations especially on cultivar B and F.

A combination of onion (*A. cepa*) extracts and farmyard manures has a negative effect on the symbiotic ants associated with aphids infesting *S nigrum*.

6.2 Recommendations

There is need for further research on onion-based botanical extracts that can be combined with farmyard manure that can reduce populations of symbiotic ants associated with aphids that produce dew while infesting *S. nigrum* under field conditions. For example, there could be onion varieties that have better insecticidal extracts than the one used.

Further research is needed to determine if the increase in pest infestations when onion extract was used could be due to a different pesticide resistant strain of the pest.

REFERENCES

- Adebooye, O.C. and Opabode, J.T. (2004) *Status of conservation of the indigenous leaf vegetables and fruits of Africa. African Journal of Biotechnology*, 3(12): 700-705.
- Agarwala, B.S and Ghosh, M.R. (1985) *Biogeographical considerations of Indian Aphidae (Homoptera)*. New Series 31: 81-96
- Akhtar Y. R. Yeoung M. B. Isman (2007) *Comparative bioactivity of selected extracts from Meliaceae and some commercial botanical insecticides against two noctuid caterpillars, Trichoplusia ni and Pseudaletia unipuncta. Phytochem Rev* (2008) 7:77–88 DOI 10.1007/s11101-006-9048-7
- Alan MacLeod (2008) *Central science laboratory. Pest risk analysis for Tetranychus evansi. Sand Hutton, York YO41 ILZ, UK.*
- Altieri Miguel A. Clara I. Nicholls (2003) *Soil fertility management and insect pests: harmonizing soil and plant health in agroecosystems Soil & Tillage Research* 72 203–211
- Amtmann A, Troufflard S, Armengaud P. (2008) *The effect of potassium nutrition on pest and disease resistance in plants. Physiol Plant*, 133(4):682-91
- Arnault I. Vey F. Fleurance C. Nabil H. Auger J. (2008) *Soil fumigation with Allium sulfur volatiles and Allium by-products. 16th IFOAM Organic World Congress, Modena, Italy June 16 -20.*

- Ben-David, T. S. Melamed, U. Gerson and S. Morin (2007) *ITS2 as barcodes for identifying and analyzing spider mites (Acari, Tetranychidae)*. *Experimental and Applied Acarology* 41 169-181.
- Blackman R. L and Eastop V. F (2006) *Aphids on the world's herbaceous plants and shrubs*. Vols. 1 and 2. John Wiley and Sons.
- Brisson, A. J. Davis, G.K. (2008) *Pea Aphid. Faculty publications in the biological sciences*. Paper 77.
- Catherine Nicholas (2007) *The most extreme bugs*. John Wiley and sons. P. 61 ISBN 978-07879-8663-6.
- Chandrashekhar Jagtap, Rajeshree Patil, Prajapati PK (2013) *Brief review on therapeutic potentialsof Kakamachi (Solanum nigrum Linn.)*. *Ayurpharm Int J Ayur Alli Sc.*, No.2 pp22-32.
- Crystal D. Grover, Kathleen C. Dayton, Sean B. Menke 1and David A. Holway (2008) *Effects of aphids on foliar foraging by Argentine ants and the resulting effects on other arthropods*. *Ecological Entomology* (2008), 33, 101–106
- Daniel H. Gillman (2005) *Sooty mold*. University of Massachusetts Amhherst.
- Denise Lim and John Pickering (2008) *Formicidae – Ants*. University of Georgia, Athens.
- Dik A.J. and J.A. van Pelt (1992) *Interaction between phyllosphre yeast, aphid honeydew and fungicide effectiveness in wheat under field conditions*. *Plant Pathology* 41(6)661-675.

Edmonds J.M. and Chweya, J.A. (1997) *Black nightshades: Solanum nigrum L. and related species: promoting the conservation and use of underutilized and neglected crops*. Rome, Italy: International Plant Genetic Resources Institute (IPGRI).

Edmonds J.M. Chweya J.A. (1997) *Black Nightshades. Solanum nigrum L. and Related Species*. (Gatersleben, Germany: Institute of Plant Genetics and Crop Plant Research/Rome: International Plant Genetic Resources Institute).

FORMAT, (2003) *Forum for Organic Resource Management and Agricultural Technologies*. Organic Resource Management, Chapter 17

Foster R.F., Obermeyer J. (2010) *Vegetable insects. Managing insects in the home vegetable garden*. Purdue extension.

Fréchette B. M. Bejan, É. Lucas, P. Giordanengo and C. Vincent (2010) *Resistance of wild Solanum accessions to aphids and other potato pests in Quebec field conditions*. *Journal of Insect Science*: Vol. 10 | Article 16/
www.insectscience.org 1

George C. McGavin (1993) *Bugs of the world*. Infobase Publishing. ISBN 0-8160-2737-4.

Gohole Linnet S, Steve Yeninek, Silvia A. Omasaja, Simon Kiarie, Elizabeth Omami, Pamela Obura, Steve Waller, Jim Simon, Maria Marshall (2013) *Sustainable African Indigenous Vegetable Production and Market –Chain Development for Improved Health and Nutrition and Income Generation by Smallholder Farmers in Kenya, Tanzania and Zambia*. Hort; CRISP; USAID.

- Gregg A, Howe and Andreas Schaller (2008) *Direct defenses in plants and their induction by wounding and insect herbivores*. Springer science and business media B.V. pp7.
- Hammond-Kosack KE, Parker JE. (2003) *Deciphering plant-pathogen communication: Fresh perspectives for molecular resistance breeding*. Curr Opin Biotechnol. 14:177-193.
- Hannan T. Reynolds and Tom Volk (2007) *Sporias spongiosa, the beach aphid poop eater*. Tom Volks fungus of the month. Wisconsin – La Crosse.
- Hasan, M.R. Ahmad, M. Rahman, M.H. Haque, M.A. (2009) *Aphid incidence and its correlation with different environmental factors*. J. Bangladesh Agril. Univ. 7(1): 15-18
- Hauser, T.P. Christensen, S. Heimes, C. Kiaer, P. (2013) *Combined effects of arthropod herbivores and phytopathogens on plant performance*. Functional Ecology 27, 623-632
- Heron et al., (2005) *Insecticide Resistance Management in cotton aphid (Aphis gossypii) and cotton mite (Tetranychus urticae)*. Cotton Research and Development Cooperation.
- Herron, G., Rophail, J. and Wilson. (2004) *Resistance monitoring in two-spotted mite: cotton seasons 2001/2002 and 2002/2003*. In: (Crop Protection) “Quality Cotton” – A Living Industry. 12th Australian Cotton Conference 10 – 12th August 2004, Gold Coast Convention and Exhibition Centre, CD ROM.
- Hoffmann. H, Learnonth S, Wood P. (2004) *Common insect pests and diseases on vegetables in the home garden*. Bulletin No. 4624.

- Linda M. Hooper-Bui (2008) “*Ant*”. World book encyclopedia. ISBN 978-0-0108-1.
- Luong Minh Chau and K.L Heong (2005) *Effects of organic fertilizers on insect pest and diseases of rice*. Cuullong Delta Rice Research Institute Co Do, Can Tho, Vietnam International Rice Research Institute. Omonrice 13: 26-33.
- Marava P. (2012) *Host Discrimination by the Green Peach Aphid (Myzus persicae Sulzer) Complex and Relative Importance of Non-Colonizing Aphid Species in the Transmission of Bushy-top and PVY in Tobacco*. A thesis for Master of Science degree in Tropical Entomology. Department of Biological Sciences University of Zimbabwe.
- Marschner H, (1995) *Mineral nutrition of higher plants*. Academic Press. London, 2nd ed. 185, 454-455.
- McCain, Christy M; and Grytnes, John-Arvid. (2010) *Elevational gradients in species richness*. In: Encyclopedia of life Science (ELS). John Wiley & Sons, Ltd: Chichester
- Mochiah, M.B., P.K. Baidoo and M. Owusu-Akyaw, (2011) *Influence of different nutrient applications on insect populations and damage to cabbage*. J. Adv. Biosci., 38: 2564-2572.
- Nagappan R. (2012) *Impact of Melia azedarach Linn (Meliaceae) dry fruit extract, farmyard manure and nitrogenous fertilizer application against aphids (Brevicoryne brassicae Linn.)*. (Homoptera,Aphididae) in home garden. *Assian Journal of Agricultural Sciences*. 4(3): 193-197.

- Naluyange V. (2014) *Compatibility of Rhizobium inoculants with water hyacinth manure formulations in common bean and consequences on Aphis fabae and Colletotricum lindemuthianum infestations.*
- Nelson Enrique Casas-Leal, Franco Alirio Vallejo-Cabrera, and Edgar Iván Estrada-Salazar (2013) *Mechanisms of resistance to Neoleucinodes elegantalis (Guenée) in wild germplasm of the genus Solanum.* *Agronomía Colombiana* 31(2), 153-160,
- Okole, B.N. and Odhav, B. (2004) *Commercialisation of plants in Africa. South African Journal of Botany, Special issue*, 70(1): 109-115.
- Patriquin, D.G., Baines, D and Abboud, A. (1995) *Diseases, pests and soil fertility.* In *Soil Management in Sustainable Agriculture*, Edited by HF Cook & HC Lee, Wye College Press, Wye, England, pp.161-174.
- Pompon Julien, Dan Quiring, Philippe Giordanengo & Yvan Pelletier (2010) *Role of host-plant selection in resistance of wild Solanum species to Macrosiphum euphorbiae And Myzus persicae.* *Entomologia Experimentalis et Applicata* 137: 73–85.
- Prasannath K. and S. Mahendran (2013) *Efficacy of botanicals on the control of cowpea pests.* Proceedings of the International Conference of Eastern University, Sri Lanka.
- Raja Nagappan (2012) *Impact of Melia azedarach Linn. (Meliaceae) Dry Fruit Extract, Farmyard Manure and Nitrogenous Fertilizer Application against Cabbage Aphid Brevicoryne brassicae Linn. (Homoptera: Aphididae) in Home*

Garden. *Asian Journal of Agricultural Sciences* 4(3): 193-197, 2012 ISSN: 2041-3890

Sanford LL, Deahl KL, Sinden SL, Ladd TL. (1992) *Glycoalkaloid contents in tubers from Solanum tuberosum populations selected for potato leafhopper resistance*. *Am potato J.* 69:693-703.

Sarwar M. (2012) *Effects of potassium fertilization in population build up on rice stem borers (lepidopteran pests) and rice (Oryza sativa L.) yield*. *Journal of cereals and oil seeds*. Vol.3 (1) pp 6-9.

Schippers, R.R. (2000) *African indigenous vegetables: an overview of the cultivated species*. Chatham, U.K: Natural Resources Institute/ACP-EU Technical Centre for Agricultural and Rural Cooperation.

Schippers, R.R. (2002) *African indigenous vegetables: an overview of the cultivated species*. Rev. ed. [CD-ROM]. Chatham, UK: Natural Resources Institute/ACP-EU Technical Centre for Agricultural and Rural Cooperation.

Sithanantham et al. In: MOA Onyango et al. (2003) *Proceedings of Third Workshop on Sustainable Horticultural Production in the Tropics*. 26-29 November 2003, Maseno University, Kenya (Maseno University, Kenya, 2003) 37-42.

Stoll, G. (2000) *Natural Crop Protection in the Tropics* Margraf Verlag. Weikersheim p.172

Suganthy M. and P. Sakthivel (2012) *Efficacy of botanical pesticides against major pests of black nightshade, Solanum nigrum Linn*. *International Journal of Pharma and Bio Sciences* 3(3): (B) 220 - 228

Thatcher LF, Anderson JP, Singh KB (2005) *Plant defense responses: What have we learnt from Arabidopsis?* *Funct Plant Biol.* 32:1-19.

Timothy M. Spann and Arnold W. Schumann (2010) *Disease and Pest Resistance / Soil Fertility and Plant Nutrition.* *Plant Biology*

Timothy M. Spann and Arnold W. Schumann (2013) *Mineral Nutrition Contributes to Plant Disease and Pest Resistance.*

Vijayalakshmi, K. Subhashini, B. Koul, S. (1999) *Plants in Pest Control: Garlic and onion.* Centre for Indian Knowledge Systems, Chennai, India pp. 30-32

Yi Sun, Ying Zhao, Lei Wang, Hongxiang Lou, Aixia Cheng (2012) *Cloning and expression biosynthesis from Solanum nigrum.* 6(5):242-248.