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Infestation and damage of black nightshade (*Solanum nigrum*) by black bean aphid (*Aphis fabae*) and red spider mite (*Tetranychus evansi*)

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Abstract

The black nightshade, *Solanum nigrum* Linn. (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 with the improved cultivars that are being adopted in western Kenya. 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 study was conducted within Masinde Muliro University of Science and Technology farm, in Kakamega County, western Kenya. The experiment was a randomized block design. Two cultivars of *S. nigrum* var. *scabrum* Linnaeus and one cultivar of *S. nigrum* var. *nigrum* Linnaeus were used. 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. Application of onion extracts reduced pest populations, and cultivars F and M tended to have the best effect.

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Introduction

The black nightshade (*Solanum nigrum* Linnaeus), 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 is 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.

Materials and methods

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 034° 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 1.

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 40cm x 20cm. 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.

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 4cmm (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.

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 (85g) were added to vegetable oil (50mL) (Golden Fry, BIDCO Kenya Ltd, Nairobi). The mixture was allowed to stand for 24 hours, then 1mL 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 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	

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.

Data collection

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.

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 ×10 magnifications using a dissecting microscope (Leica ZOOM 2000, Model Z45E, Leica Inc., Buffalo, NY U.S.A.)

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 multtes.

Results

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, χ^2=1.10; p<0.05) (Table 2) *S. nigrum var. nigrum* cultivar B grown with chicken manure had the highest aphid population; *S. nigrrm 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 3)

Table 2. 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 ≤ 0.001 ** P ≤ 0.01 * P ≤ 0.05

In trial 2, there were significant interactions between cultivars, manure types and the sprays on aphid populations. (df=17; χ^2=374.96; p < 0.05) (Table 4). 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 3. Mean number of aphids (*Aphis fabae*) collected per plant.

Source of variation	Cultivar	Treatment	Means of number *Aphis fabae*
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	Chi- Square
Cultivar x manure x spray		17	1019.52***

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

The 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.

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; χ^2=1019.52; $p<0.05$) (Table 5). 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.

Table 4. Mean number of mites collected per plant.

Sourse of variation	Cultivar	Treatment	*Tetranychus evansi*
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	*Tetranychus evansi*
Cultivar*manure*Spray		17	374.96***

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

Plant Biomass of the three cultivars

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 5. Mean dry weights per plant in grams.

Source of variation	Cultivars	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 ≤ 0.001, ** P ≤ 0.01, * P ≤ 0.05

Discussion

Infestations by aphids and red spider mites

Aphids and red spider mites varied between nightshade cultivars. *Solanum nigrum var. nigrum* (Cultivar B) had the highest population of *A. fabae* when planted without manure treatments. *Solanum nigrum var. scabrum* (Cultivar M) from the open market had the lowest *A. fabae*. 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.

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. *Aphis fabae* 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 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. This suggests that cultivar B is tolerant to the pests but less productive.

Conclusions

Based on the present study, it was 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*.

Competing interests

Authors have declared that no competing interest exists.

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