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Efficacy of onion (*Allium cepa*) extracts on the black bean aphis (*Aphis fabae*) and red spider mite (*T. evansi*) on black nightshade (*S. nigrum*) cultivars when planted with different manure regimes

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Abstract
Black nightshade, *Solanum nigrum* Linnaeus (Solanaceae), is a tropical plant used as a nutritive vegetable and herbal medicine, it expresses high levels of secondary metabolites such as steroidal glycoalkaloids and phytoalexins which offer protection against pests and microbial pathogens. However, 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. The current research is aimed at developing an integrated pest management approach based on *Allium cepa* Linnaeus extracts and farmyard manures to control the aphid *A. fabae* Linnaeus and a mite *T. evansi* Linnaeus infesting improved *S. nigrum* Linnaeus cultivars in western Kenya. The experiment was a randomized block design. Data was analyzed using SAS version 9.1. Onion extracts reduced pest populations and cattle manure had significant positive effects.

Keywords: *Solanum nigrum*, *Tetranychus evansi*, *Allium cepa*, *Aphis fabae*, *Tetranychus evansi*

Introduction
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* [1, 2]. They are consumed as leafy vegetables, are sources of indigenous fruits and for medicinal purposes [2-4]. *S. nigrum* species are among the most common and highly important leafy vegetables in the warm and humid zones of Africa [5].
Two groups of arthropods, namely class insecta and class arachnida attack black nightshades and other solanaceous plants [6]. Insect pests that attack nightshades and other solanaceuos 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 [6, 7]. They cause mottled leaves, yellowing and stunted growth, curled leaves, browning and reduced yields on the host plants [8, 9]. 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 [10, 11]. 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 [12-14].
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 [6]. The red spider mite is a polyphagous mite and feeds on wild and cultivated solanaceae, especially tomatoes, potatoes, black nightshades and tobacco [15]. The pest also feeds on a number of non-solanaceous plants like beans, citrus, cotton, tea, coffee and castor beans [15]. Damage by red spider mites causes leaves to become chlorotic with the formation of white to brown spots twisted leaves and low productivity [16].
Red spider mites are identified by formation of spider webs and the formation of white to brown spots. Plant breeders and biotechnologists have been developing improved cultivars that

have less bitterness [17].

However, such improved less bitter cultivars express low levels of pest-inhibitive compounds, and hence exhibit reduced resistance to pests and pathogens [18, 19, 20]. 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 [8,21,22,23]. Development of organic solutions to pests and soil fertility problems in the cultivation of improved varieties of *S. nigrum* Linnaeus is therefore necessary [19, 24- 27].

2. Materials and Methods

2.1 Study site

A field study was conducted within the facilities of the Masinde Muliro University of Science and Technology farm, in Kakamega County, western Kenya (latitude N 00 17.104', longitude E 034^0 45.874'; altitude 1561m a.s.l.) [19]. The study area has two rainy 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 [19]. Soils in this area are loamy with the properties described (Table. 1).

Table 1: Experimental plot soil composition [19].

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	

2.2 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.

2.3 Planting material

Seeds of *S. nigrum* var. *scabrum* (A) 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 (Fig. 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.

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 (Fig. 2).

Fig 1: A healthy *S. nigrum var scabrum*

Fig 2: Mature *S. nigrum var scabrum*

2.4 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 [18], 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.

2.5 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 at 7-day intervals [12]. The controls were sprayed at the same time with distilled water.

2.6 Data collection

2.6.1 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 a weekly basis to determine the rate of plant growth. Plants with deformed leaves were recorded.

2.6.2 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 ×10 magnifications using a dissecting microscope (Leica ZOOM 2000, Model Z45E, Leica Inc., Buffalo, NY U.S.A.)

2.7 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 lsd means when the ANOVA was significant. Data on aphid and mite populations were analyzed using proc genmod (Poisson) and means separated using proc multtes

3. Results and Discussions

3.1 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 [9] (Fig. 3). Mites were identified as *Tetranychus evansi* (red spider mites) were identified based on body size, shape and tarsal setae [1] (Fig. 4).

Fig 3: Apterous *Aphis fabae* Linnaeus

Fig 4: *Tetranychus evansi* Linnaeus

3.2 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, 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. 2)

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. 3). 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 mixed with chicken manure. This was followed by those without manure application while plants mixed 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. 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 Cultivar x Manure	df 8	Chi- Square 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 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 Cultivar x manure x spray		df 17	Chi- Square 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.

3.3 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; χ^2=1019.52; p<0.05) (Table. 4). 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 plant 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: Mean number of mites collected per plant

Source **of variation**	**Cultivar**	**Treatment**	**Means of *Tetranychus rvansi***
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 x manure x spray		17	374.96***

Treatment means followed by the same letter within a particular column are statistically not different. $P\leq0.05$.

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 populationsin 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.

5. Conclusion

The number of aphids was highest in cultivar *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 *Solanum nigrum* var. *nigrum* Linnaeus, showing a preference that occurs during the presence of non-preferred hosts. *S. nigrum var. scabrum* from University Farm and *S. nigrum var. scabrum* from market, tended to have the best effect. Application of onion extracts reduced pest populations.

6. 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.

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