ESSENTIAL OIL COMPOSITION AND PHYTOTOXICITY EFFECTS OF *SOLANUM NIGRUM* L. ON SEED GERMINATION OF SOME VEGETABLES

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Abstract. The allelopathic activities of *Solanum nigrum* shoot and root aqueous extracts on cabbage, spinach and tomato seed germination were investigated. Treatment concentrations were 0, 2, 4, 6, 8 and 10 mg ml^{-1}. The results indicate that the shoot extracts did not affect the germination of tomato and spinach seeds but 2 mg ml^{-1} slightly hindered the germination of cabbage seeds. The highest root extract concentration (10 mg ml^{-1}) led to a significantly lower germination percentage in tomato seeds in comparison with the control. The highest extract concentration (10 mg ml^{-1}) in both shoot and root extracts slowed down germination time in tomato seeds. The mean germination time indicates that cabbage seeds germinated faster than tomato and onion. These results indicate that *S. nigrum* shoot and root extracts pose no hindering effect on the germination of cabbage and onion seeds, although increased root extract concentrations may hinder germination in tomato seeds. A total of 16 and 12 compounds were respectively identified from the shoots and roots of *Solanum nigrum* analysed by GC-MS. The major shoot compositions were Citronellol (11.98%), Cyclohexanol, 2-methyl-5-(1-methylethenyl) (5.84%), Geraniol (3.76%) and Geranyl tiglate (3.53%). In the roots, Hexacosane (35.62%), 1H-Indole, 1-methyl-2-phenyl (12.66%), Hexadecanal (8.11%) and Tetrasiloxane, decamethyl (7.58%) were the major components.
Keywords: *allelopathy, wild vegetable, seed viability, chemical compositions, GC-MS*

Introduction

The domestication of wild vegetables as a means of combatting food insecurity has been a topic of interest in South Africa for over a decade. Various aspects of wild vegetables have been widely documented including their agronomy and more commonly their nutritional compositions (Edmonds and Chweya, 1997; Odhav et al., 2007; Flyman and Afolayan, 2008; Atta et al., 2010; Mahala et al., 2012). Although the cultivation of wild vegetables for food is common in some African countries such as Botswana, Nigeria, Ghana, Zambia and Zimbabwe, this practice is not widespread in South Africa (Gbile et al., 1988; Mushita, 1997; Madisa and Tshamekang, 1997; Maroyi, 2011; Aju et al., 2013). Since wild vegetables are commonly viewed as weeds, intercropping them with conventional crops and/or vegetables is one of the options that need to be explored in order to make maximum use of the land in the fight against food insecurity. However, there are fears that wild vegetables may cause growth problems or nutritional stress to exotic vegetables growing alongside them.

The stimulatory or inhibitory effects plants have on the growth of other plants within the same ecosystem through a mechanism known as allelopathy is well known. Bio-chemicals known as allelochemicals/allelochems are released from plant parts by leaching, root exudation, volatilisation, residue decomposition and other processes in both natural and agricultural systems (Fraenkel, 1959; Stamp, 2003). The

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allelochemicals are secondary metabolites like terpenoid and phenolic compounds such as flavonoids, anthocyanins, lignin as well as tannins and these have specific actions (Khanh et al., 2007). In recent years, there have been interests in the allelopathic activities of wild plants. Ataollahi et al. (2014) reported that increasing the concentration of the *Eucalyptus globulus* leaf extract had a strong allelopathic effect on *S. nigrum* seed germination, shoot and root length growth. In another study, Sadeghi et al. (2010) reported that the water extract of *Helianthus annuus* inhibited the germination of *S. nigrum* seeds. The roots of *Tithonia diversifolia* have been found to stimulate the growth and chlorophyll content of some *Solanum* species (Otusanya et al., 2014). Sabh and Ali (2010) found *S. nigrum* extracts to hinder dicotyledonous seedling growth and chlorophyll in comparison with monocotyledonous seeds. *S. nigrum* has also been reported to reduce germination, root and shoot length growth of onion (Baličević et al., 2015). Some allelochemicals such as alkaloids that are produced by *S. nigrum* have been found responsible for suppressing growth of other plants (Sabh and Ali, 2010).

Although *S. nigrum* is an important wild vegetable of the Eastern Cape Province, its allelopathic activity on some important vegetables has not been investigated. Cultivating wild vegetables alongside traditional vegetables in farming operations is an option being explored in order to reduce food insecurity. The current experiment was therefore conducted to investigate the effect of *S. nigrum* root and shoot aqueous extracts on the germination of cabbage, spinach and tomato seeds. Understanding plant on plant interactions is important in agricultural production especially in relation to intercropping. The detrimental effects of inclusion of wild vegetables into the existing farming operation on the ecology, environmental degradation and conservation of genetic biodiversity require a better understanding. This study was therefore conducted to help know the stimulatory or inhibitory effects of the residues of *S. nigrum* on the germination of these important traditional vegetables. Also, a comprehensive understanding of the essential oil composition of *S. nigrum* is vital for proper understanding of its value as both a nutritional and pharmacological plant. The essential oils of the fresh roots and shoots were therefore also analysed for their chemical compositions.

Materials and methods

Plant collection

S. nigrum plant samples were previously identified in 2011 at the University of Fort Hare and the voucher specimen (BVE 11/017) kept at the Giffen herbarium. Fresh samples were collected by uprooting the whole plant from around Alice in August 2015 in the wild and washing with distilled water to remove impurities. Cabbage (Starke Ayres - Cabbage: Drumhead), tomato (Starke Ayres- Hybrid Tomato: STAR 9003) and spinach (Starke Ayres - Swiss Chard: Fordhook Giant) seeds were purchased from a local supermarket.

Preparation of aqueous extracts

The method of Badmus and Afolayan (2012) was used to prepare the extracts of the plant samples. The root and shoot samples were separately dried to a constant weight in the oven at 35°C. The dry matter yields were ground in an electric hammer mill fitted with 1 mm sieve and stored separately in tightly sealed vial bottles in the refrigerator at

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4°C till further use. About 50 g of each powdery material was extracted in 1.5 L of distilled water and agitated on an orbital shaker for 12 h at room temperature. The filtrate was freeze dried at -50°C under the vacuum (RVT4104, USA) and reconstituted in distilled water to obtain the desired concentrations of 10, 8, 6, 4 and 2 mg ml^{-1}.

Viability test

Viability tests were conducted using the Tetrazolium Chloride Technique. Following the method described by Peters (2000), 25 seeds were imbibed in water overnight at 22.5 ± 2.5°C in triplicates. The seeds were then cut along the margin without damaging the embryo and soaked in colourless 0.1% solution of 2,3,5- triphenyltetrazolium chloride (TTC) for 24 h at 22.5 ± 2.5°C in the dark. The pH of the TTC solution was 6.61. The seeds were removed from the TTC solution, washed in distilled water and soaked in 95% ethanol to permit direct observation of the embryo under the microscope. Embryos of viable seeds appeared reddish in colour.

Seed germination trials

To evaluate the effects of the five shoot and root aqueous extract concentrations on the vegetable seeds, the method of Badmus and Afolayan (2012) was adopted with modifications. In triplicates, 25 seeds of each vegetable were placed evenly in 9 cm Petri dishes lined with two Whatman filter papers and moistened with the respective extract concentrations and distilled water in the control. The petri dishes were laid out in a Randomised Complete Block Design on a laboratory work bench under ambient temperature conditions (between 19 and 25°C). The seeds were examined on a daily basis and considered germinated when the radicle was visible.

Gas chromatography mass spectrometry (GC-MS)

Using the modified method of Okoh and Afolayan (2011), 100 g of the fresh leaf and root samples of *S. nigrum* were subjected to a DRYDIST Milestone manufactured (2007) Solvent-free Microwave Extraction (SFME) Labstation apparatus for 30 min. The extracted oil was kept in tightly sealed vial bottles in a refrigerator at 4°C till further use. GC-MS analyses were performed on the Agilent 5977A MSD and 7890B GC System, Chemetrix (pty) Ltd; Agilent Technologies, DE (Germany) with a Zebron-5MS column (ZB-5MS 30 m x 0.25 mm x 0.25 um) (5%-phenylmethylpolysiloxane) apparatus. The column and temperature conditions which were used were as follows: GC grade helium at a flow rate of 2 ml/ min and splitless 1 µl injections were used. The oven temperature, injector and source temperatures were set at 70, 280 and 280°C respectively. The ramp settings were; 15°C/ min to 120°C, then 10°C/ min to 180°C, then 20°C/ min to 270°C and held for 3 min.

Identification of components

The identification of the chemical constituents of the essential oil was determined by their GC retention times, percentage composition (Area %) and retention indices. The interpretation and identification of their mass spectra was confirmed by mass spectral incorporated library. The identification was further confirmed by search using the National Institute of Standards and Technology (NIST) database (NIST/EPA/NIH mass spectral library 2014) and comparing with those of published data.

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Statistical analysis

Where applicable, the data were subjected to statistical analysis using MINITAB Release 12.22. A one way analysis of variance was used to compare seed germination as influenced by the extract concentration. Means were compared using Duncan's multiple range test. The means were treated as significantly different at $p < 0.05$.

Results and discussion

Seed viability and germination

Viability in the purchased cabbage, spinach and tomato seeds was 99, 92 and 83% respectively. In *S. nigrum* (belonging to the same family as tomatoes) seeds stored at room temperature in a period ranging from 1 to 9 years, Roberts and Lockett (1978) reported 99, 100, 96, 98, 91, 73, 27, 2 and 0% viability. This indicates an inverse relationship between seed viability and time. However, the viability and germination results are in close agreement.

The effects of the aqueous extract of *S. nigrum* shoot on seed germination of cabbage; tomato and spinach are shown in *Table 1*.

Table 1. *Percentage inhibition on germination of vegetable seeds as influenced by the aqueous extract of S. nigrum shoot extract*

	Vegetable seeds		
	Cabbage	**Tomato**	**Spinach**
10 mg ml^{-1}	97 ± 0.58^{ab}	83±2.08	65±3.06
8 mg ml^{-1}	97 ± 0.58^{ab}	92±1.73	80±1.00
6 mg ml^{-1}	100 ± 0.00^{a}	92±0.58	64±2.00
4 mg ml^{-1}	100 ± 0.00^{a}	99±0.58	72±4.58
2 mg ml^{-1}	96 ± 0.00^{b}	89±0.79	68±3.61
Control	100 ± 0.00^{a}	95±2.31	79±1.16

Values shown are MEAN ± SD
Different letters down the same column represent significant differences at $p < 0.05$

The results indicate that the shoot extract did not significantly ($p < 0.05$) affect the germination of tomato and spinach seeds. However, 2 mg ml^{-1} significantly lowered the germination of cabbage seeds although this was not significantly lower than 10 mg ml^{-1}, the highest concentration. The root extract of *S. nigrum* did not significantly affect the germination of cabbage and spinach seeds but significantly hindered the germination of tomato seeds (*Table 2*).

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Table 2. *Percentage inhibition on germination of vegetable seeds as influenced by the aqueous extract of S. nigrum root extract*

	Vegetable seeds		
	Cabbage	**Tomato**	**Spinach**
10 mg ml^{-1}	98±0.58	13±1.73[b]	42±1.16
8 mg ml^{-1}	96±0.58	87±1.73[a]	80±1.73
6 mg ml^{-1}	96±0.58	62±4.62[a]	60±3.31
4 mg ml^{-1}	98±0.58	80±2.00[a]	44±3.06
2 mg ml^{-1}	100±0.00	87±0.00[a]	40±4.58
Control	100±0.00	84±1.53[a]	56±5.03

Values shown are MEAN ± SD
Different letters down the same column represent significant differences at $p < 0.05$

The highest concentration (10 mg ml^{-1}) significantly lowered tomato seed germination. The Mean Germination Time (*Figs. 1* and *2*) indicates that cabbage seeds germinated faster than spinach and tomato seeds. However, 10 mg ml^{-1} of both extracts significantly slowed down tomato seed germination time. Numerous allelopathic effects of *S. nigrum* on germination and growth have been performed on a wide range of crops and scantily on some important horticultural crops such as tomato, cabbage and onion. For example, Agarwal et al. (2002) reported that *S. nigrum* strongly inhibited plumule growth by 100% and radicle growth by 92% in some wheat cultivars. In the same study, *S. nigrum* strongly inhibited seedling growth as compared to other plants. *S. nigrum* extracts have also been reported to hinder the growth of chickpea as well as some tomato and onion cultivars (Kadioglu et al., 2004). Girija et al. (2008) as well as Marinov-Serafimov (2010) also reported some allelopathic effects of *S. nigrum* on some legumes. Although, studies on other species indicate that *S. nigrum* strongly hinders the growth of some important species, the present results indicate that this plant does not pose the same effects on some important horticultural crops such as tomato, cabbage and spinach.

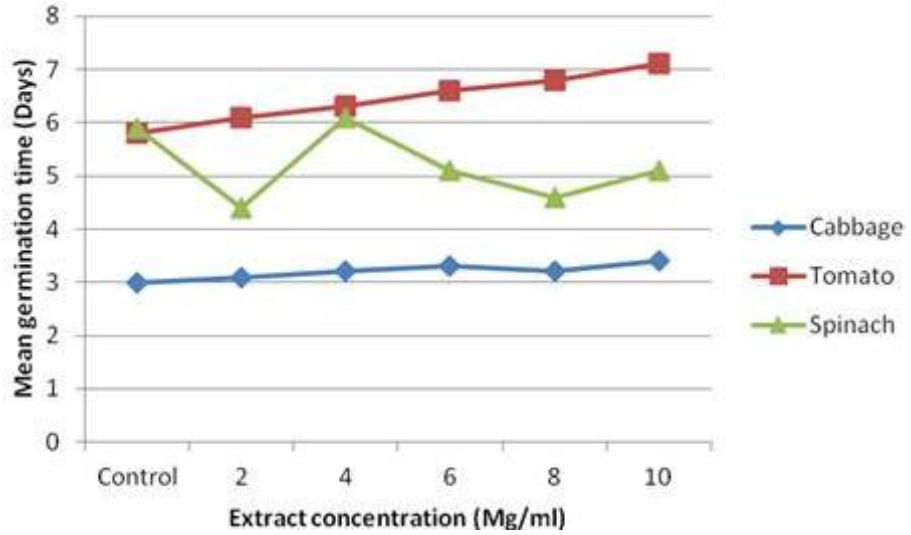

Figure 1. *Effect of S. nigrum aqueous shoot extract on seed germination time of vegetables*

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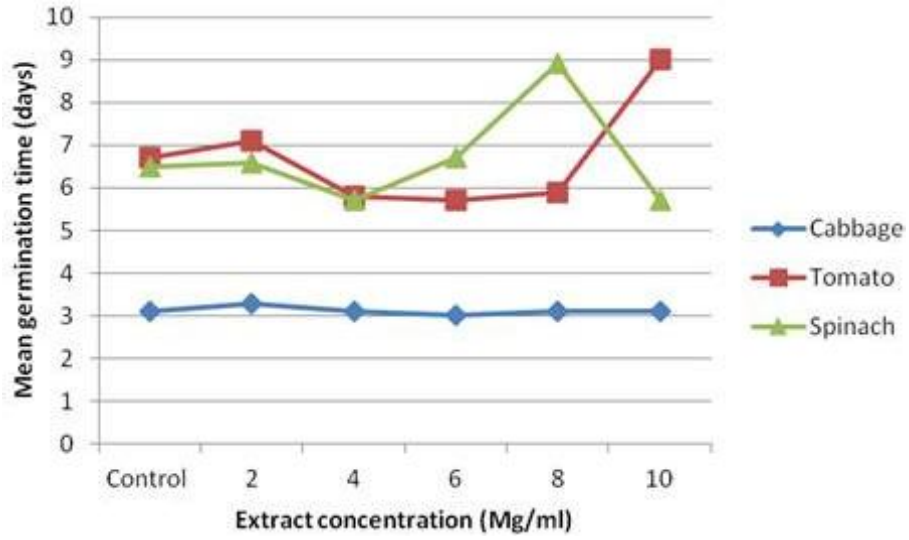

Figure 2. *Effect of S. nigrum aqueous root extract on seed germination time of vegetables*

Essential oil compositions

Both the shoot and root samples yielded a colourless oil with a pungent scent after extracting with the microwave. A total of 16 compounds were identified from the shoot oil extract (*Table 3*) and 12 were identified from the root oil extract (*Table 4*).

Table 3. *Essential oil chemical composition of S. nigrum shoots*

Name	Molecular formula	RT	KI	Area (%)
α-farnesene	$C_{15}H_{24}$	8.085	1580	2.57
.beta.-iso-Methyl ionone	$C_{14}H_{22}O$	8.779	1673	2.63
1-Benzothiazol-2-yl-3-(4-methyl-benzoyl)-thiourea	$C_{16}H_{13}N_3OS_2$	7.893	2334	0.93
2-[2-Thienyl]-4-acetyl quinoline	$C_{15}H_{11}NOS$	7.736	1545	0.75
3-(4-Fluoro-phenyl)-4-methyl-2-(2, 2,2-trifluoro-1-methoxycarbonyl-ethylidene)-2,3-dihydro-thiazole-5-carboxylic acid ethyl ester	$C_{17}H_{15}F_4NO_4S$	9.121	1596	2.02
Benzamide, 4-methoxy-N-[4-(1-methylcyclopropyl)phenyl]-	$C_{18}H_{19}NO_2$	10.955	1729	0.66
Chola-5,22-dien-3-ol, (3.beta.,22Z)-	$C_{24}H_{38}O$	8.477	-	1.91
Citronellol	$C_{10}H_{20}O$	6.249	1368	11.98
Cyclobutanecarboxylic acid, 2-phenylethyl ester	$C_{13}H_{16}O_2$	8.717	1690	2.71
Cyclohexanol, 2-methyl-5-(1-methylethenyl)-	$C_{10}H_{18}O$	11.766	1920	5.84
Cyclopentaneethanol, 2-(hydroxymethyl)-.beta.,3-dimethyl-	$C_{10}H_{20}O_2$	8.242	1567	0.98
Geraniol	$C_{10}H_{18}O$	6.452	1388	3.76
Geranyl isobutyrate	$C_{14}H_{24}O_2$	7.936	1594	1.36
Geranyl tiglate	$C_{15}H_{24}O_2$	9.318	1703	3.53
p-Anisaldehyde 4-[1-adamantyl]-3-t hiosemicarbazone	$C_{19}H_{25}N_3OS$	8.845	1506	1.92
Silane, chlorodimethyloctadecyl	$C_{20}H_{43}CLSi$	8.440	1534	0.87

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In the shoots, the major compositions were Citronellol (11.98%), Cyclohexanol, 2-methyl-5-(1-methylethenyl) (5.84%), Geraniol (3.76%) and Geranyl tiglate (3.53%). In the roots, Hexacosane (35.62%), 1H-Indole, 1-methyl-2-phenyl (12.66%) and Hexadecanal (8.11%) were the major components. Four compounds, *viz*, α-farnesene, Citronellol, Geraniol and Geranyl tiglate were identified in both the shoot and root extracts. Although some authors (Ogundajo et al., 2013; Sivakamasundari et al., 2013; Taherpour, et al., 2013; Aburjai et al., 2014; Huda et al., 2015) have reported various compounds in the leaves of *S. nigrum*, none have reported the findings of this current work. Aliero et al. (2006) reported the presence of Geraniol in the leaves of *Solanum pseudocapsicum* and this corresponds with what was found in both the shoot and root extracts of the present project.

Table 4. *Essential oil chemical composition of S. nigrum roots*

Name	Molecular formula	RT	KI	Area (%)
1,2,4-Benzenetricarboxylic acid, -dodecyl dimethyl ester	$C_{23}H_{34}O_6$	13.008	-	0.43
1H-Indole, 1-methyl-2-phenyl-	$C_{15}H_{13}N$	14.360	2207	12.66
2-Phenylethyl tiglate	$C_{13}H_{16}O_2$	8.711	1689	1.83
α-farnesene	$C_{15}H_{24}$	8.081	1579	2.38
Citronellol	$C_{10}H_{20}O$	6.249	1368	11.98
Eicosane	$C_{20}H_{42}$	12.191	1910	2.78
Geraniol	$C_{10}H_{18}O$	6.452	1388	3.76
Geranyl tiglate	$C_{15}H_{24}O_2$	9.318	1703	3.53
Hexacosane	$C_{26}H_{54}$	11.305	-	35.62
Hexadecanal	$C_{16}H_{32}O$	9.380	1672	8.11
Nonadecane	$C_{19}H_4O$	10.335	1655	4.45
Phthalic acid, 2-(1-adamantyl)ethyl methyl ester	$C_{21}H_{26}O_4$	8.774	-	2.24

Conclusion

The current study indicates that the viability and germination results are in close agreement. The shoot extract hindered the germination of cabbage while the root extract hindered that of tomato. Also, an increase in the shoot and root extract concentration slowed down the germination time of tomato seeds. However, the results indicate that *S. nigrum* does not pose a severe hindering effect on the germination of cabbage, spinach and tomato and as such possibilities of cultivating the wild vegetable alongside these exotic vegetables remain open. *S. nigrum* shoots and roots also possess a diverse range of chemicals in their essential oils. A total of 16 and 13 compounds were respectively identified by GC MS in the shoot and root extracts of the plant.

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