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ALLELOPATHIC ACTIVITY OF NIGHTSHADE (*Solanum nigrum* L.) ON SEEDLING GROWTH OF CERTAIN WEEDS AND CROPS

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ABSTRACT

The allelopathic activity of nightshade, *Solanum nigrum* L. extract and residue on the seedling growth and chlorophyll content of certain weeds and crops was investigated. Obtained results indicated that shoot length of London rocket and root length of sowthistle were the most sensitive plants based on I_{50} and I_{90} values to the aqueous extract of nightshade, whereas wheat was the least sensitive plant. Nightshade residue was more phytotoxic to dicotyledonous plants than monocotyledonous. Moreover, wheat and onion crops have greater tolerance than londonrocket and sowthistle weeds. Addition of 2% (w/w) of dried nightshade leaves to 500 g soil severely reduced the seedling length and dry weight of sowthistle seedling by 21.6 and 16.8% of the control, respectively beside occurrence of complete inhibition (100%) for sowthistle growth at a higher rates (4 and 8%, w/w). Also, chlorophyll a and b contents in the dicotyledonous plants were decreased with increasing the residue, in contrast to the monocotyledonous plants which were not affected. Allelochemicals in nightshade leaves were identified as alkaloids such as atropine, hyoscine and solanine in addition to capric, myristic, palmitic and oleic fatty acids. These results suggest that nightshade may have allelopathic activity and that alkaloids and fatty acids may be responsible for the growth reduction or inhibition in the affected plants.

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INTRODUCTION

Chemical interference with plant growth is termed allelopathy. Allelopathy is characterized by inhibition in plant emergence or growth. Chemicals which have allelopathic activity are called allelochemicals. If present in low enough concentrations, they may stimulate rather than inhibit growth **(Putnam, 1988)**. Allelochemicals vary from simple molecules such as ammonia and ethylene to the complex compounds such as fatty acids, flavonoids and alkaloids. Allelopathic effect of plant extracts and their residual activity in soil on weeds and crops was investigated by many workers **(Abdallah *et al* 2002; El-Mutlaq *et al* 2002; Iqbal *et al* 2003; Norsworthy, 2003; El-Khatib *et al* 2004; Shukla and Singh, 2004; Ali, 2005; Travis *et al* 2006; Erin *et al* 2007 and Virender *et al* 2009)**. The objective of the present work was to study the allelopathic effect of aqueous extract and residue rates of nightshade *Solanum nigrum* L. on the seedling growth and chlorophyll content of five crops and weed plants in addition to isolate and identify allelochemicals present in nightshade.

MATERIALS AND METHODS

1- Plant used

Nightshade, *S. nigrum* L. is a summer annual broadleaf weed and it is a member of the solanaceae family that are known to produce allelochemicals. This weed was obtained from Experimental station, Faculty of Agriculture, Cairo University, Giza to study its biological effect on seedling growth and chlorophyll content of five plant species as shown in **Table (1)**.

Table 1. English, scientific and family names of the tested species

English name	Scientific name	Family
Wheat	*Triticum aestivum* L.	Gramineae
Onion	*Allium cepa* CV.Giza 20	Alliaceae
Sowthistle	*Sonchus oleraceus* L.	Compositae
London rocket	*Sisymbrium irio* L.	Cruciferae
Lettuce	*Lactuca sativa* L.	Compositae

2. Preparation of crude extract

100g of air-dried leaf of *S.nigrum* were ground in a grinder and were extracted with distilled water at the rate of 2 ml/g plant material as described by **Su and Horvat (1981)**. After 24 hours, the extract was filtered through Whatman No.3 filter paper and eliminated water to dryness under vacuum at-50°C using Lioalfa 6-50 Freeze Dryer apparatus. The crude extract was kept in the refrigerator till it was assayed.

3. Bioassay technique

3.1. Laboratory tests

Twenty five seeds of each tested species (wheat, onion, sowthistle, London rocket and lettuce) were placed on Whatman No. 1 filter paper in a 10cm diameter glass Petri dish. Four concentrations of crude extract, 25, 50, 100 and 200 ppm were added to each Petri dish (5ml distilled water/Petri dish). In the control only distilled water was added. Four replicates were made for each treatment. The seeds of sowthistle, London rocket and wheat were incubated at 25°C, whereas onion and lettuce at 20°C. The root and shoot lengths were measured after ten days of incubation. Inhibition percentages were calculated as [(control-treatment)/control] x 100 and were analyzed using a probit method **(Finney, 1971)** to estimate the concentration required to inhibit 50% and 90% (I_{50} and I_{90}) of seedling growth (root and shoot lengths).

3.2. Glasshouse tests

Five glasshouse tests were done in plastic pots (12 cm diameter) at Faculty of Agriculture, Cairo University during the season of 2007/2008. Ground black nightshade foliage material was mixed with a constant weight of soil at the rates of 5, 10, 20 and 40 g per 500 g soil (W/W) per pot, with ratio 1, 2, 4 and 8%. Ten seeds of each tested species were placed in each pot, 0.5 cm below the soil surface and then were irrigated with tap water as needed. A completely randomized design with four replicates was used. The seedling length was measured after 35 days of treatment. Seedling samples were then oven dried at 80°C for 48h and weighed to estimate their dry weights. The concentration of chlorophyll a and b (Mg/g fresh weight) were determined according to **Welburn and Lichtenthaler (1984).**

4. Isolation and identification of allelochemicals

4.1. Alkaloids

Aqueous extract of nightshade foliage was separately chromatographed on Whatman No. 1 paper alongside with the authentic samples using the solvent system: ethanol (70%): Amonium hydroxide (25%), 99:1 (V/V). Ascending development technique was used and the chromatogram was air dried then sprayed with the Dragendorff's as chromogenic agent to help identifying alkaloids according to **Zweig and Sherma (1972).** Rate of flow (R_f values) for each compound and the authentic samples were calculated.

4.2. Fatty acids

The methyl esters of the fatty acids were analyzed by GLC apparatus, using Pye-Unicam column-PEGA, 10%. Program was: 70°C (Initial temp.) with a rate of 5°C/min. to 190°C (Final temp.). Detector temp. 300°C. injection temp. 250°C flow rate of gasses: N_2: 30 ml/min. H_2: 33 ml/min. Air : 330 ml/min.

The identification of the fatty acids was achieved by comparing the retention time (R_t) of their peaks with those of the authentic samples under the same conditions. The relative percentage of each compound was determined using triangulation method according to **Nelson *et al* (1969).**

RESULTS AND DISCUSSION

1. Laboratory tests

Data in **Tables (2 and 3)** indicate that shoot length of London rocket and root length of sowthistle were the most sensitive plants to the aqueous extract of nightshade with I_{50} values of 42.9 ppm for London rocket shoot length and 32.1 ppm for sowthistle root length. Whereas, wheat was the least sensitive plant with I_{50} values of 165.3 and 118.6 ppm for shoot and root lengths, respectively. In general, root length of the tested plants were more sensitive to the extract than shoot length. The five tested plants can be arranged descendingly according to their sensitive based on I_{50} values to the extract as follows for shoot length: London rocket (42.9 ppm), sowthistle, (59.3 ppm), lettuce (84.0 ppm), onion (107.6 ppm) and wheat (165.3 ppm). Also, the I_{90} values to the extract gave a similar trend being 93.0, 105.8, 253.4, 304.5 and 381.2 ppm, respectively. In the same connection, the five tested plants can be arranged in descending order according to the sensitivity of their root length based on I_{50} values as follows sowthistle (32.1 ppm), lettuce (37.0 ppm), London rocket (47.5 ppm), onion (78.6 ppm) and wheat (118.6 ppm). Sensitivity to the extract followed the same order according to the I_{90} values of 64.2, 75.2, 112.8, 265.4 and 424.6 ppm, respectively.

2. Glasshouse tests

2.1. Seedling growth

Data presented in **Table (4)** reveal that all the tested crops and weeds showed inhibition sensitivity to the nightshade soil residue. Although some were more sensitive than others, seedling growth of dicotyledonous plants exhibited greater sensitivity to the residue than monocotyledonous Moreover, in dicotyledonous plants sowthistle weed was more sensitive than lettuce crop. Addition of 2% (W/W) of dried nightshade leaves to 500 g soil reduced the seedling length of lettuce, London rocket and sowthistle by 83.0, 40.4 and 21.6% as well as decreased the seedling dry weight of the same plants by 96.2, 35.0 and 16.8% of the control, respectively. Moreover, nightshade soil residues exerted complete inhibition (100%) for sowthistle growth at a higher rates (4 and 8%, W/W). In contrast to this, wheat and onion growth were not affected by residues at 1 to 4% (W/W), in soil whereas at 8% (W/W), seedling length of wheat and onion were reduced by 80.6 and 72.1% of the control, respectively.

Interpretation for these results were previously reported by **Fayed *et al* (1997),** who found that allelochemicals liberated in soil from weed roots by physical secretion or exudation and by microbial decomposition seems to have inhibitory impact on division and elongation of meristemic cells in developed roots and consequently reduced growth parameters of both neighboring and following crop plants.

2.2. Chlorophyll content

The results given in **Table (5)** show that chlorophyll "a" and "b" contents in wheat and onion leaves after five weeks of planting were not obviously affected by nightshade leaves soil residues at all used rates.

At 2% (W/W) chlorophyll contents in lettuce, London rocket and sowthistle leaves were reduced by 85.0, 61.0 and 47.5% for chlorophyll "a" and by 96.2, 68.7 and 42.0% for chlorophyll "b", respectively. Also, nightshade residue at 4% (W/W) decreased chlorophyll "a" and "b" to 75.2 and 93.5% for lettuce leaves. This decrease was 32.0 and 50.4% for London rocket leaves at the same rate. It is worthy to notice that the depressing effect of nightshade soil residue on chlorophyll content of leaves of tested plants, was more pronounced in dicotyledonous plants, than monocotyledonous ones. With all, chlorophyll "a" and "b" contents in the dicotyledonous plants were gradually decreased with increasing the concentration of nightshade residue in soil.

In our studies, although a limited number of dicotyledonous and monocotyledonous plants were evaluated, there appeared to be differences in sensitivity between the two groups. Broadleaf weeds such as London rocket and sowthistle were more sensitive to the nightshade residue than monocotyledonous crops such as wheat and onion. Such trend gives a competitive advantage to monocotyledonous crops over dicotyledonous weeds in addition to possibility broadleaf weeds control which grow in monocotyledonous crops by selective toxicity of nightshade.

Table 2. Effect of nightshade aqueous extract on shoot length of certain weeds and crops

Test species	Conc. (ppm) of nightshade extract % Inhibition in shoot length				I_{50} (ppm)	I_{90} (ppm)	Slope ± S.E.
	25	50	100	200	95% confidence limits		
L. rocket	17.9	62.0	90.3	100	*(38.6) 42.9 **(47.3)	*(81.2) 93.0 **(110.9)	3.82±0.34
Sowthistle	0.0	43.5	83.0	100	*(33.4) 59.3 **(103.7)	*(71.2) 105.8 **(471.3)	5.09±0.89
Lettuce	0.0	40.6	60.0	79.0	*(----) 84.0 **(-----)	*(----) 253.4 **(----)	2.67±0.75
Onion	0.0	19.1	58.2	70.2	*(32.0) 107.6 **(149.0)	*(137.5) 304.5 **(516.2)	2.84±0.66
Wheat	0.0	0.0	32.0	56.8	*(----) 165.3 **(----)	*(----) 381.2 **(----)	3.53±0.90

* Lower limit ** Upper limit

Table 3. Effect of nightshade aqueous extract on root length of certain weeds and crops

Test species	Conc. (ppm) of nightshade extract % Inhibition in root length				I_{50} (ppm)	I_{90} (ppm)	Slope ± S.E.
	25	50	100	200	95% confidence limits		
Sowthistle	35.1	74.0	100	100	*(28.6) 32.1 **(35.4)	*(56.5) 64.2 **(76.4)	4.26±0. 45
Lettuce	28.6	59.8	100	100	*(0.46) 37.0 **(87.2)	*(45.9) 75.2 **(104.3)	4.17±0.93
L.Rocket	16.2	57.6	81.3	100	*(42.5) 47.5 **(52.6)	*(99.0) 112.8 **(136.4)	3.41±0.29
Onion	10.7	34.0	60.1	82.5	*(69.0) 78.6 **(90.1)	*(210.3) 265.4 **(366.5)	2.43±0.23
Wheat	4.7	19.5	49.0	66.0	*(102.7) 118.6 **(140.7)	*(316.0) 424.6 **(652.0)	2.31±0.24

* Lower limit ** Upper limit

Table 4. Effect of nightshade leaves residues on the seedling growth of some weeds and crops

Residue rate	Wheat		Onion		Lettuce		L. rocket		Sowthistle	
	Seedling length	Seedling dry weight	Seedling length	Seedling dry weight	Seedling length	Seedling dry weight	Seedling length	Seedling dry weight	Seedling length	Seedling dry weight
(%,W/W)	% of control									
0	100	100	100	100	100	100	100	100	100	100
1	94.2	116.0	101.0	105.0	90.4	99.0	66.1	62.7	58.0	49.8
2	91.2	110.0	93.4	105.2	83.0	96.2	40.4	35.0	21.6	16.8
4	88.0	103.1	86.5	97.0	74.0	81.3	19.7	10.5	0.0	0.0
8	80.6	97.5	72.1	89.3	59.0	70.6	0.0	0.0	0.0	0.0

Table 5. Effect of nightshade leaves residues on chlorophyll a and b contents of some weeds and crops

Residue rate	Wheat		Onion		Lettuce		L. rocket		Sowthistle	
	Chl.a	Chl.b	Chl.a	Chl.b	Chl.a	Chl.b	Chl.a	Chl.b	Chl.a	Chl.b
(%,W/W)	% of control									
0	100	100	100	100	100	100	100	100	100	100
1	106.3	108.2	99.2	112.0	89.0	102.1	75.3	89.0	70.0	83.0
2	100.7	105.6	101.2	104.3	85.0	96.2	61.0	68.7	47.5	42.0
4	100.0	102.0	93.1	104.0	75.2	93.5	32.0	50.4	0.0	0.0
8	90.0	'94.6	88.5	91.7	60.4	82.0	0.0	0.0	0.0	0.0

The present results are in line with those obtained by **El-Mutlaq *et al* (2002)** who found that the dicotyledonous plants (alfalfa and wildradish) were more sensitive to the alkaloidal extract of *Rhazya stricta* than the monocotyledonous plants (ryegrass and wheat). They added that root length was more sensitive than shoot length in all tested plants. Moreover, **Chen *et al* (1997)** reported that annual wormwood (*Artemisia annua*) residue was more phytotoxic to small-seeded broadleaf weeds such as pigweed and lambsquarters than large-seeded crops such as corn and soybean. In the same connection, **Norsowrthy (2003)** mentioned that broadleaf weeds, sicklepod and pricklysida appeared a higher sensitive to the aqueous extract and residue of wildradish than monocotyledonous crops, wheat and corn. He showed that detrimental effect of the wildradish on broadleaf weeds would alter the competitive relationship between crops and weeds towards crop plants.

3. Identification of allelochemicals

3.1. Alkaloids

Data in **Table (6)** reveal that leave extract of nightshade contained atropine, solanine and hyoscine compounds with R_f values of 0.39, 0.86 and 0.94, respectively. These fractions showed positive reactions when sprayed with the dragendroff's reagent to give orange color on the chromatogram whereas two spots with R_f values of 0.10 and 0.52 gave negative reactions using the same reagent indicating the absence of alkaloids.

Table 6. Identification of alkaloids present in foliage extract of nightshade

R_f values	Alkaloids	Reaction
0.10	Unknown	(-)
0.39	Atropine	(+)
0.52	Unknown	(-)
0.86	Solanine	(+)
0.94	Hyoscine	(+)

(-) = negative reaction
(+) = positive reaction

3.2. Fatty acids

It is clear from **Table (7)** that leave extract of nightshade contained capric, lauric , myristic and palmitic as saturated fatty acids and also the presence of oleic and linoleic as unsaturated fatty acids. The GLC analysis revealed that palmitic acid represents the higher percentage of fatty acid (24.19%) followed by linoleic acid (17.22%) and then myristic acid (14.03%). Meanwhile, the relative percentages of oleic, capric and lauric acids were 13.70, 11.26 and 8.53%, respectively.

Table 7. Identification of fatty acids and their relative percentages present in foliage extract of nightshade

No. of carbon atom	Fatty acids	Relative percentage (%)
10:0	Capric	11.26
12:0	Lauric	8.53
14:0	Myristic	14.03
16:0	Palmitic	24.19
18:1	Oleic	13.70
18:2	Linoleic	17.22
-	Unknown	6.84
-	Unknown	3.92

These results are in agreement with those obtained by **Rizvi *et al* (1987)**, whom found that the purine alkaloids (theophylline, paraxanthine and caffeine) extracted from *Coffee Arabica* L. leaves demonestrated allelopathic activity. They calculated the litter leaves might release 1 to 2g caffeine/m^2 each year. **Lovett *et al* (1981)** mentioned that the tropane alkaloids, hyoscyamine and scopolamine isolated from leaves *Datura stramonium* L. exhibited allelopathic effect on lettuce seedlings at concentrations of less than 50 ppm. **Similarly, Tsuzuki *et al* (1987)** indicated that rice seedling growth was inhibited by palmitic, stearic and arachidic acids separated from buckwheat. These fatty acids also may cause buckwheat to be allelopathic to other plants. In the same trend, **Ali (2005)** showed that aqueous extract and residue of *Cyperus rotundus* had allelopathic effect on the germination and growth of *Echinochloa crus-galli* and *Phalaris minor.* Allelopathic inhibitors identified as flavonoid and phenolic compounds such as rutin, kaempferol and quercetin flavonoids as well as P.hydroxybenzoic, chlorogenic and ferulic phenolic acids.

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حوليات العلوم الزراعية
جامعة عين شمس ، القاهرة
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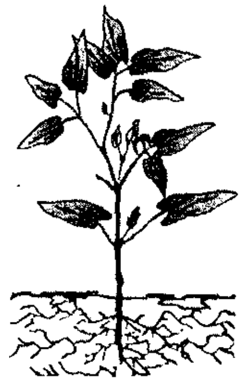

النشاط الاليلوباثى لحشيشة عنب الديب على نمو بادرات بعض الحشائش والمحاصيل

[٧]

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الموجز

اجرى هذا البحث لدراسة تأثير المستخلص المائى من اوراق حشيشة عنب الديب ومتبقياته على نمو بذور خمس انواع من المحاصيل والحشائش وهى : القمح - البصل- الخس- الحارة - الجعضيض وكذلك فصل وتعريف المركبات ذات التأثير الاليلوباثى لمستخلص عنب الديب باستخدام التحليل الكروماتوجرافى (الورقى- الغازى) ومواد قياسية. أظهرت النتائج ان المستخلص المائى لعنب الديب كان اكثر تأثيراً على طول الجذير لجميع النباتات المختبرة عن طول الريشة، وكان طول الريشة لنبات الحارة وطول الجذير لنبات الجعضيض من أكثر اجزاء النباتات المختبرة حساسية للمستخلص، حيث كانت قيمة I_{50} هى ٤٢,٩، ٣٢,١ جزء فى المليون لهم على التوالى. وعموما كان الترتيب التصاعدى فى أطوال الريشة على اساس قيمة I_{50} للمستخلص هى:الحارة - الجعضيض - الخس - البصل - القمح، فى حين كان الترتيب التصاعدى فى اطوال الجذير هى: الجعضيض - الخس - الحارة - البصل - القمح.

فيما يتعلق بمتبقيات عنب الديب فى التربة، وجد ان بقايا أوراق عنب الديب فى التربة تؤثر على نمو جميع النباتات المختبرة مع حدوث تثبيط كامل لنمو نبات الجعضيض من المعدلات العالية ٨,٤% (وزن/وزن)، ولوحظ ان تأثير متبقيات اوراق عنب الديب على نمو النباتات ذات الفلقتين مثل الخس كان اكثر وضوحا من النباتات ذات الفلقة الواحدة مثل البصل، كذلك كانت الحشائش عريضة الاوراق مثل الجعضيض والحارة، اكثر حساسية لمتبقيات أوراق عنب الديب من المحاصيل ذات الفلقة الواحدة مثل القمح والبصل مما يعطى، ميزة تنافسية لصالح الاخيرة بالاضافة الى امكانية الاستفادة من هذا التأثير الاختيارى لعنب الديب فى مكافحة الحشائش العريضة الاوراق النامية فى المحاصيل ذات الفلقة الواحدة.

من ناحية اخرى لم يتأثر المحتوى الكلوروفيلى فى أوراق القمح والبصل بمتبقيات عنب الديب على جميع التركيزات المختبرة (وزن/وزن)، وعلى العكس من ذلك إنخفض المحتوى الكلوروفيلي فى اوراق نباتات الخس والجعضيض والحارة بزيادة تركيز المتبقى من عنب الديب فى التربة.

وأوضحت النتائج ايضا ان المستخلص المائى لأوراق عنب الديب، يحتوي على ثلاثة مركبات قلويدية وهى الاتروبين والهيوسين والسولانين بجانب وجود الاحماض الدهنية مثل الكابريك والميرستيك والبالمتيك والأوليك، وقد تكون هذه المركبات هى المسئولة عن النشاط الاليلوباثى لعنب الديب على نمو النباتات المختبرة.

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