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Allelopathic effects of methanolic extracts of potato (*Solanum tuberosum* L.) varieties and isolated compounds on *Avena fatua* L. and *Brassica campestris* L. Weeds in Qinghai-Tibet plateau

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ABSTRACT

In first part of this study, the potato variety Q9 was found the most allelopathic variety. Therefore, in present study chemical analysis of variety Q9 was done and 7 compounds [1- (2, 6-Dihydroxy-4-methoxy-3-methylphenyl) ethanone (**I**), Gentiopicroside (**II**), β-sitosterol (**III**), palmitic acid (**IV**), kaempferol (**V**), verbascoside (**VI**), and isoverbascoside (**VII**) were isolated by column chromatography and identified by structural analysis. Compounds **I**, **II**, **VI** and **VII** were present in methanolic extracts. These compounds stimulated the seedlings growth of *A. fatua*, however, but inhibited the seedlings growth of *B. campestris.* Hence, we presumed that these compounds in potato variety Q9, may control the *B. campestris* weed, hence, more in-depth research is needed.

Keywords: Allelopathic effects, aqueous extracts, *Avena fatua*, *Brassica campestris*, flowering, leaves, rape, *Solanum tuberosum*, stems, tubers, tuberisation, varieties, weeds, wild oat

INTRODUCTION

The plant lacks the ability to move, hence, they have developed alternate defence strategies, using their secondary metabolites for pests control and to overcome the biotic and abiotic stresses (cold, heat, frost and drought etc.). Besides the secondary metabolites also protects the plants from the harmful effects of UV light etc. (11,16,22). Inhibitory allelopathic effects (1) are the negative effects of allelochemicals (produced and released from the plants) on the growth and development of other susceptible plant species. Weeds cause great yield losses in crops through release of allelochemicals resulting into their dominance in any area (4,7,17,18).

Potato (*Solanum tuberosum* L.) is one of the main crops grown in Qinghai Province of China and has recently become the fourth main food in China. The detrimental effects of weeds on potato crop are very serious problem and very few herbicides are available for weed control in potato crop (10) giving rise to need for biological weed control method. Plants impact the neighbouring plants through release of allelochemicals in the environment, but the allelopathic effects of potato on weed species have not been reported. This study aimed to identify the allelochemicals present in potato Q9 variety and to assess their role in weed control. The allelopathic effects of methanolic extracts from Q9 and X65 varieties and compounds isolated from the allelopathic Q9 variety, were determined on

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germination and seedlings growth of *Avena fatua* L. and *Brassica campestris* L. weeds. This information may help to find the allelopathic effects of potato variety and understand their underlying mechanisms. The findings of this study may possibly help in the development of new environmental-friendly herbicides to control wild oat and rape weeds in potato crop.

MATERIALS AND METHODS

These studies were done at the Qinghai Academy of Agriculture and Forestry Sciences in Qinghai Province, China (36°43′N; 101°45′E, at 2230 m above sea level). The 4potato (*Solanum tuberosum* L.) varieties [Qingshu 2 (Q2), Qingshu 9 (Q9), Xiazhai 65 (X65) and175] samples were collected at early, flowering, tuberisation growth stages from Tibet Plateau Biotechnology Key Lab of Ministry of Education. These varieties were sown in April and harvested in September 2015. The seeds (80-100 % germination) of wild oat (*Avena fatua* L.) and rape (*Brassica campestris* L.) were purchased from the local market.

(I). Bioassays with methanolic extracts

Experimental treatments consisted of 4 factors: (i). Potato varieties: 2 (Q9 andX65), (ii). Plant parts: 2 (Shoot and underground), (iii). Methanolic extracts concentrations: 6 (0,2,5,10,20,50 mg/ml) and (iv). weed species : 2 (*A. fatua*, *B. campestris*). The treatments were replicated thrice in completely randomized Design.

Sample collection: Samples of shoots (leaves and stems) and underground-part (roots, tubers and soil samples attached to the roots) of potatovarieties Q9 and X65 were collected at 3-leaf stage (1 month after planting).The collected samples were shade dried at room temperature (20°C) for 7 days; thereafter, cut into 3 cm long pieces and 100 g pieces were soaked in 100 ml MeOH for 7 days. After filtering, the MeOH extract was concentrated in vacuo to yield residue, which was dissolved in distilled water and filtered through filter paper. The methanolic extract solutions of 2, 5, 10, 20 and 50 mg/ml concentrations were prepared. The methanolic extract solutions were filtered through 0.22 µm Millipore filter and stored at 4 ℃ for tests.

The effects of methanolic extracts of potato varieties Q9 and X65were assessed on the germination and seedlings growth of *A. fatua* and *B. campestris* weeds in petri-plate bioassay. All Petri-dishes (9 cm dia) were sterilized at 121°C for 20 min in an autoclave. The test seeds were sterilized by soaking in 5% NaClO solution for 10 mins and then washed thrice with sterile water. Sixteen uniform sterilized seeds of *A. fatua* and 20 seeds of *B. campestris* were sown equidistant in each Petri dish lined with filter paper. Distilled water was used as control. Six ml extracts or distilled water was added per petri dish. These petri-dishes were kept in an incubator at 24°C, with afluorescent light of intensity $356 \pm 0.16 \times 10^3$ lux for 8 h. Seed germination and seedling growth (root length, shoot length and dry weight) of the weeds were determined 7 days after incubation (1).

(II). Isolation, identification and bioactivity of compounds in potato variety Q9

Extraction and isolation of compounds: The whole plant of potato variety Q9 was collected at early vegetative stage (One month after planting)and its methanolic extract was prepared as previously described. The water-soluble fraction was then desalted and the Dianion-HP20 column was eluted with distilled water. Then, the fraction was isolated by HPLC-2030, eluted with MeOH-H_2O (60:40). Afterwards, the fraction was subjected to silica gel (H, 10-40 µm), MCI-gel CHP20P (75-150 mm), Sephadex LH-20 gel (25-100 µm) and HPLC analysis gel.

HPLC analysis: The fraction was subject to ODS C18 and eluted with C_2H_3N-H_2O (90:10) to give compounds **I**, **II** and **III,** eluted with C_2H_3N-H_2O (80:20) to give the compound **IV.** The fraction was also subject to XAqua C18 and eluted with C_2H_3N-H_2O (20:80) to give compound **V,** eluted with C_2H_3N-H_2O (32:68) to give compound **VI** and eluted with C_2H_3N-H_2O (30:70) to give compound **VII**.

Bioassay with isolated compounds

Experimental treatments consisted of 3factors: (i). Isolated compounds: 7 (I,II,III,IV,V,VI,VII), (ii). Isolated compounds concentrations: 6 (0,1,2,5,10,25 mg/ml) and (iii).Weed spp.: **2** (*A. fatua, B. campestris*). The treatments were replicated thrice in completely randomized design. These 7- isolated compounds were dissolved in 1 % v/v DMSO to prepare the1, 2, 5, 10, 25 mg/ml concentrations. The distilled water with 1% v/v DMSO was used as control. The effects of these 7isolated compounds from potato variety Q9 were tested on the seed germination and seedlings growth of *A. fatua* and *B. campestris* weeds. Seed germination and seedling growth (root length, shoot length, fresh weight and dry weight) of recipient plants were determined 7 days after incubation (1).

Statistical analysis: The root lengths, shoot lengths, dry weight and inhibition rate were subjected to one-way ANOVA analysis of variance followed by Duncan's multiple-range test to determine significant differences among mean values at the probability level of 0.05 (4). All bioassays were performed three times under the identical conditions. Data are presented as means ± SE.

RESULTS AND DISCUSSION

A. BIOASSAYS (METHANOLIC EXTRACTS)

The methanolic extracts of potato varieties Q9 and X65inhibited the seeds germination of both *A. fatua* and *B. Campestris* weeds (Fig. 1). However, the magnitude of inhibitory effects of crude extracts of potato varieties Q9 and X65 were more drastic on seed germination of *B. campestris* than on *A. fatua* weed (Fig. 1).

(i). A. fatua

Germination: The crude extracts of potato Q9and X65 varieties shoots at 50 mg/ml concentration significantly inhibited(86.8% and 57.9%, respectively)the *A. fatua* weed seed germination (Fig. 1).

Root length: The crude extracts of potato varieties Q9 and X65 significantly inhibited the root length (Fig. 1). The root length inhibitions from the Q9 variety at 10, 20 and 50 mg/ml concentrations were < -78.1%. The root length inhibitions from potatoX65 variety at 10, 20, and 50 mg/ml concentrations were < -76.1%). This X65 variety tubers extracts at 20 and 50 mg/ml concentrations nearly completely inhibited (-94.1% and -96.4% inhibition respectively) the root length of *A. fatua* weed (Fig. 1).

Shoot length: For Q9 variety, the methanolic extract of shoots at 20 and 50 mg/ml concentrations significantly inhibited (-63.6%, -97.5%) the shoot length (Fig. 1). The methanolic extract of underground organs at 20 and 50 mg/ml concentrations were slightly less inhibitory (-50.0%, -94.2%) to shoot length (Fig. 1). For X65 variety, the methanolic extract of shoots at 20 and 50 mg/ml concentrations significantly inhibited (-65.4 %, -91.2 %) the shoot length (Fig. 1). The methanolic extract of underground organs at 20 and 50 mg/ml concentrations also significantly inhibited (-67.6 %, -83.4 %) the shoot length (Fig. 1). Thus, the effects of methanolic extracts of both shoots and underground organs of Q9 variety were more inhibitory than X65 potato variety at 50 mg/ml.

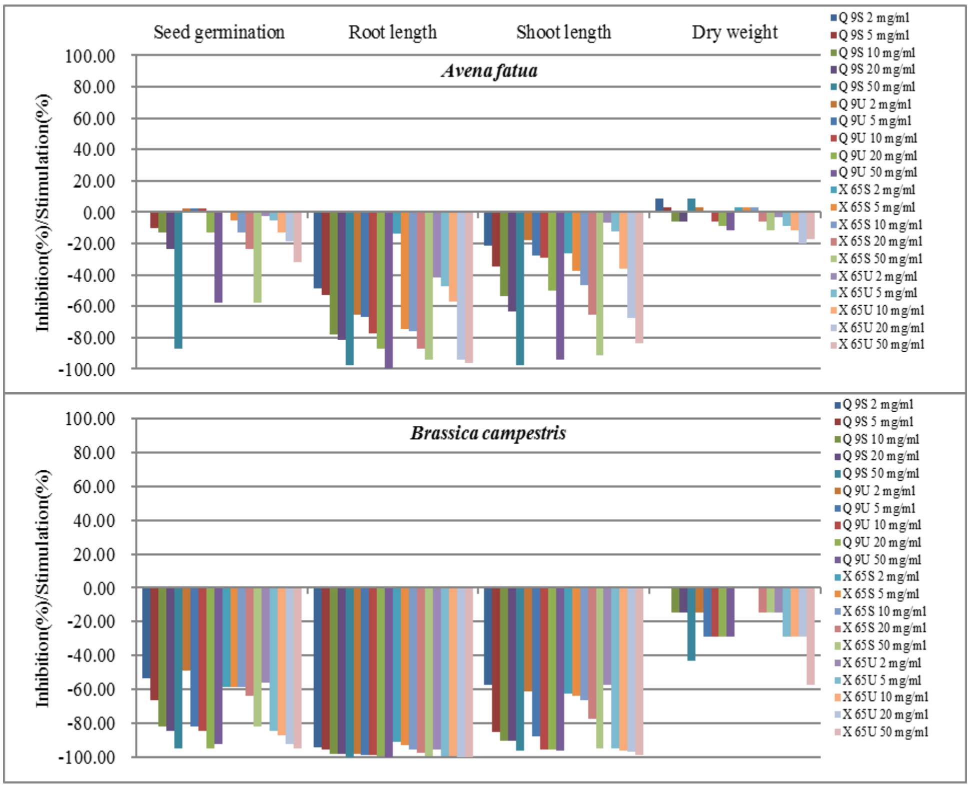


Figure 1. Inhibitory and stimulatory effects of different concentrations of methanolic extracts of potato varieties Q9 and X65 on seed germination and seedling growth of *A. fatua* and *B. campestris* weeds at 7days after sowing.
Q9S : Shoots of Q 9,Q9U : Underground organs of Q9,X65S : Shoots of X65,X65U : Underground organs of X65.

Dry weight: The effects of methanolic extracts of potato varieties Q9 and X65 were variable on the dry weight of *A. fatua*. The lower doses (2 and 5 mg/ml) of Q9S and Q9U potato varieties slightly increased the dry weight. Similarly X65 lower doses (2,5 and 10 mg/ml) also increased the dry weight of *A. fatua* (Fig. 1).

(*ii*). *B. campestris*

Germination: The crude extracts of potato varieties Q9 and X65 significantly inhibited (-60.0 % to -100.0 % respectively) the seeds germination of *B. campestris* weed (Fig. 1).
Root length: All concentrations of methanolic extracts of Q9 and X65 varieties almost completely inhibited the root length of *B. Campestris* (Fig. 1).
Shoot length: All concentrations of methanolic extracts of Q9 and X65 varieties, drastically reduced the shoot length of *B. campestris* seedlings (Fig. 1).
Dry weight: The methanolic extracts of both Q9 and X65 varieties were inhibitory to dry weight, but the magnitude of inhibition was less as compared to root and shoot length. The X65 potato variety at lower doses (2,5 and 10 mg/ml) had no effects on dry weight (Fig. 1).

In this study, the allelopathic inhibitory effects of methanolic extracts of Q9 variety were much greater than X65 variety, hence, the Q9 variety was chosen as allelopathic variety to identify and obtain its allelochemicals.

B. ISOLATION AND BIOACTIVITY OF COMPOUNDS IN POTATO VAR. Q 9

Isolation of compounds : Bioassay-guided isolation of the secondary metabolites in potato variety Q9, identified 7 known compounds [1- (2, 6-Dihydroxy-4-methoxy-3-methylphenyl) ethanone (**I**) (13), gentiopicroside (**II**) (20), β-sitosterol (**III**) (19), palmitic acid (**IV**) (9), kaempferol (**V**) (2), verbascoside (**VI**) (14), and isoverbascoside (**VII**) (20)]. These compounds were identified by comparing the ^{1}H-, ^{13}C-NMR and MS spectra with literatures (Fig. 2).

The following 7-compounds were identified:
(i). 1- (2,6-Dihydroxy-4-methoxy-3-methylphenyl) ethanone (I) : Yellow needle-shaped crystal, molecular formula $C_{11}H_{14}O_3$, ^{13}C NMR (151 MHz, CD_3OD) δ 204.2, 165.8, 164.1, 162.8, 105.9, 104.7, 91.1, 55.8, 33.0, 7.3.^{1}H NMR (600 MHz, CD_3OD) δ 6.00 (s, 1H), 3.85 (s, 3H), 2.57 (s, 3H), 1.95 (s, 3H).

(ii). Gentiopicroside (II) : White powder, molecular formula$C_{16}H_{20}O_9$, ^{13}C NMR (151 MHz, CD_3OD) δ 166.3, 150.6, 135.0, 127.0, 118.5, 117.2, 104.9, 100.2, 98.5, 78.4, 78.0, 74.5, 71.5, 70.9, 62.8, 46.6.^{1}H NMR (600 MHz, CD_3OD) δ 7.47 (s, 1H), 5.78 (ddd, *J* = 17.2, 10.4, 6.9 Hz, 1H), 5.69 (d, *J* = 3.0 Hz, 1H), 5.64 (m, 1H), 5.28 – 5.21 (overlap, 2H), 5.09 (m, 1H, H_b-7), 5.01 (dd, *J* = 17.8, 3.6 Hz, 1H), 4.67 (d, *J* = 7.9 Hz, 1H), 3.92 (dd, *J* = 12.0, 2.3 Hz, 1H), 3.67 (dd, *J* = 11.9, 6.3 Hz, 1H), 3.39 – 3.32 (overlap, 3H), 3.27 (m, 1H), 3.17 (dd, *J* = 9.2, 7.9 Hz, 1H, H-9).

(iii). β-Sitosterol (III): White powder, molecular formula $C_{29}H_{50}O$, ^{13}C NMR (151 MHz, CD_3OD) δ 142.4, 122.4, 72.5, 58.3, 57.6, 51.8, 47.4, 43.6, 43.1, 41.2, 38.6, 37.7, 37.4, 35.2, 33.3, 33.0, 32.4, 30.6, 29.3, 27.5, 25.3, 24.3, 22.2, 20.1, 19.8, 19.5, 19.4, 12.3.^{1}H NMR (600 MHz, CD_3OD) δ 5.35 (m, 1H), 3.40 (tt, *J* = 10.7, 4.9 Hz, 1H), 2.27 – 2.17 (overlap, 2H), 2.05 (dt, *J* = 12.6, 3.6 Hz, 1H), 1.98 (mj, 1H), 1.87 (dt, *J* = 13.1, 4.1 Hz, 2H), 1.79 (m, 1H), 1.69 (m, 1H), 1.63 (m, 1H), 1.59 – 1.44 (overlap, 4H), 1.42 – 1.36 (overlap, 2H), 1.35 – 1.18 (overlap, 5H), 1.18 – 1.04 (overlap, 4H), 1.03 (s, 3H), 0.96 (d, *J* = 6.5 Hz, 3H), 0.88 (t, *J* = 7.5 Hz, 3H), 0.86 (d, *J* = 6.9 Hz, 3H), 0.84 (d, *J* = 6.9 Hz, 3H), 0.72 (s, 3H).

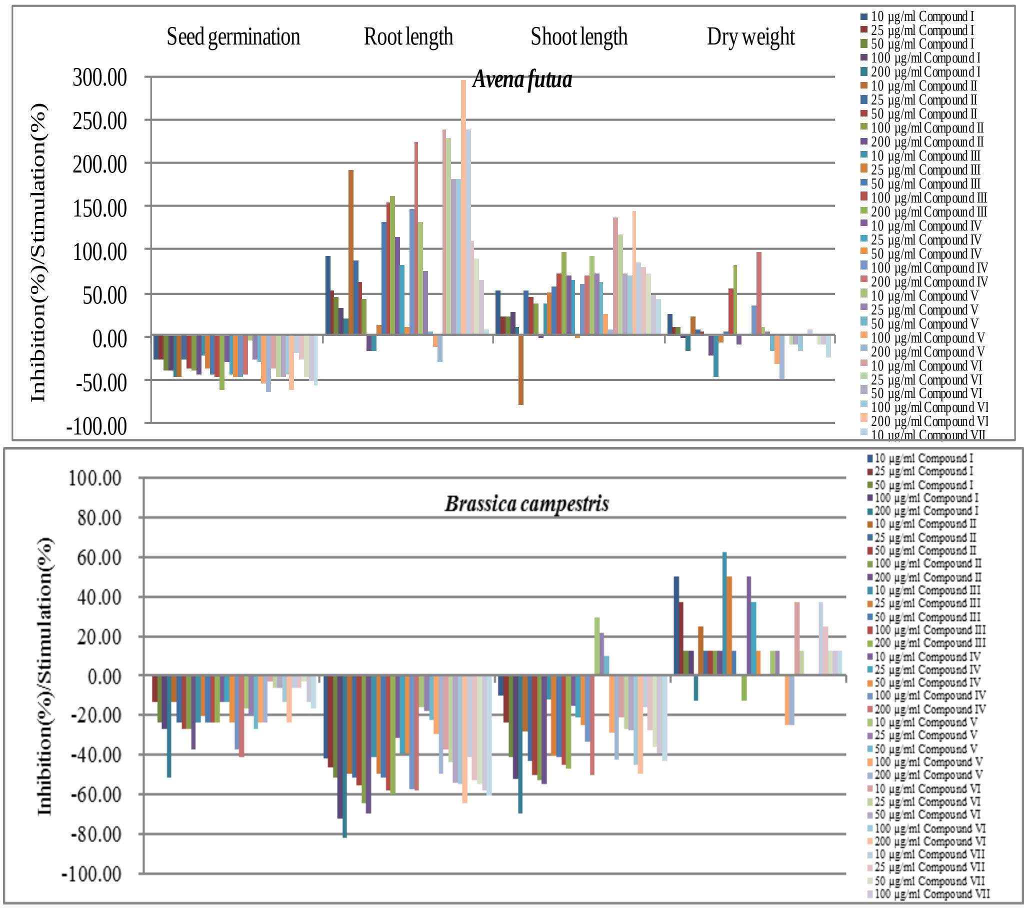


Figure 2. Inhibitory and stimulatory effects of concentrations of 7-isolated compounds isolated from potato variety Q9, on the seeds germination and seedling growth of *A. fatua* and *B. campestris* weeds at 7days after sowing.

(iv). Palmitic acid (IV): White crystal, molecular formula $C_{16}H_{32}O_2$, ^{13}C NMR (151 MHz, CD_3OD) δ 177.7, 35.0, 33.1, 30.8, 30.8, 30.7, 30.6, 30.5, 30.5, 30.3, 26.1, 23.7, 14.5.^1H NMR (600 MHz, CD_3OD) δ 2.26 (t, *J* = 7.4 Hz, 2H), 1.59 (p, *J* = 7.2 Hz, 2H), 1.29 (overlap, 24H), 0.89 (t, *J* = 7.0 Hz, 3H).

(v). Kaempferol (V): Yellow solid, molecular formula $C_{15}H_{10}O_6$, ^{13}C NMR (151 MHz, CD_3OD) δ 177.4, 165.6, 162.5, 160.6, 158.3, 148.1, 137.2, 130.7, 123.7, 116.3, 104.5, 99.3, 94.5. ^1H NMR (600 MHz, CD_3OD) δ 8.09 (d, *J* = 8.6 Hz, 2H), 6.91 (d, *J* = 8.6 Hz, 2H), 6.40 (d, *J* = 2.1 Hz, 1H), 6.19 (d, *J* = 1.9 Hz, 1H).

(vi). Verbascoside (VI): White solid, molecular formula$C_{29}H_{36}O_{15}$, ^{13}C NMR (151 MHz, CD_3OD) δ 168.3, 149.8, 148.0, 146.8, 146.1, 144.7, 131.5, 127.7, 123.2, 121.2, 117.1, 116.5, 116.3, 115.2, 114.7, 104.2, 103.0, 81.6, 76.2, 76.1, 73.8, 72.4, 72.3, 72.1, 70.6, 70.4, 62.4, 36.6, 18.4.^{1}H NMR (600 MHz, CD_3OD) δ 7.60 (d, *J* = 15.9 Hz, 1H), 7.06 (d, *J* = 2.1 Hz, 1H), 6.96 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.78 (d, *J* = 8.1 Hz, 1H), 6.70 (d, *J* = 2.1 Hz, 1H), 6.68 (d, *J* = 8.1 Hz, 1H), 6.57 (dd, *J* = 8.1, 2.1 Hz, 1H), 6.28 (d, *J* = 15.9 Hz, 1H), 5.19 (d, *J* = 1.9 Hz, 1H), 4.92 (t, *J* = 9.5 Hz, 1H), 4.38 (d, *J* = 7.9 Hz, 1H), 4.05 (m, 1H), 3.92 (dd, *J* = 3.3, 1.8 Hz, 1H), 3.82 (t, *J* = 9.2 Hz, 1H), 3.73 (dt, *J* = 9.4, 7.5 Hz, 1H), 3.63 (m, 1H), 3.60 – 3.51 (overlap, 4H), 3.39 (dd, *J* = 9.1, 8.0 Hz, 1H), 3.29 (t, *J* = 9.5 Hz, 1H), 2.84 – 2.75 (m, 2H), 1.10 (d, *J* = 6.2 Hz, 3H).

(vii). Isoverbascoside (VII) : White solid, molecular formula $C_{29}H_{36}O_{15}$, ^{13}C NMR (151 MHz, CD_3OD) δ 169.1, 149.6, 147.2, 146.8, 146.1, 144.7, 131.4, 127.7, 123.1, 121.3, 117.1, 116.5, 116.4, 115.1, 114.8, 104.4, 102.7, 84.0, 75.7, 75.4, 74.0, 72.4, 72.4, 72.3, 70.4, 70.0, 64.6, 36.7, 17.9.^{1}H NMR (600 MHz, CD_3OD) δ 7.58 (d, *J* = 15.8 Hz, 1H), 7.05 (d, *J* = 2.1 Hz, 1H), 6.91 (dd, *J* = 8.3, 2.2 Hz, 1H), 6.78 (d, *J* = 8.1 Hz, 1H), 6.68 (d, *J* = 2.0 Hz, 1H), 6.65 (d, *J* = 8.1 Hz, 1H), 6.55 (dd, *J* = 8.1, 2.1 Hz, 1H), 6.30 (d, *J* = 15.8 Hz, 1H), 5.19 (d, *J* = 1.8 Hz, 1H), 4.51 (dd, *J* = 12.0, 2.2 Hz, 1H), 4.37 (dd, *J* = 12.0, 5.8 Hz, 1H), 4.34 (d, *J* = 8.1 Hz, 1H), 4.01 (m, 1H), 3.99 – 3.94 (overlap, 2H), 3.75 – 3.70 (overlap, 2H), 3.58 – 3.52 (overlap, 2H), 3.42 (ddd, *J* = 9.5, 5.3 Hz, 2H), 2.82 - 2.77 (overlap, 2H), 1.26 (d, *J* = 6.2 Hz, 3H).

BIOASSAY (POTATO ISOLATED COMPOUNDS)

(I). *Avena fatua*

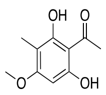
***(i).* Seeds germination:** The effects of compounds **I-VII** isolated from the potato variety Q9 on seed germination of *A. fatua* L. and *B. campestris* L. are given in Fig. 3. Only compounds **III**, **V**, **VI** and **VII** at 20µg/ml concentration inhibited the seed germination of *A. fatua* by 36.67%, 33.33%, 36.67% and 40.00%, respectively.

***(ii).* Seedlings growth:** The inhibitory effects of compounds **I-VII** isolated from potato var. Q9, were evaluated at 10, 25, 50, 100 and 200 µg/ml concentrations on root length, shoot length and dry weight of *A. fatua* (Fig. 3). All compounds had stimulatory effects.

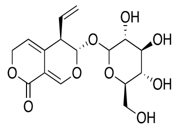
Stimulatory effects on *A. fatua*: All 7-test compounds did not inhibit its seedling growth; rather, stimulated the seedling growth (Fig. 3), especially compounds **II-VII** also stimulated the root length.

(i). Compound I: It was significantly stimulatory (+92.0 %) to the root length of *A. fatua* at 10 µg/ml concentration, but was moderately stimulatory (+50.9 %) at 20 µg/ml concentration. It also moderately stimulated (+52.6 %) the shoot length of *A. fatua* at 10 µg/ml concentration (Fig. 3).

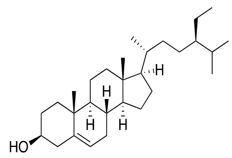
(ii). Compound II: At 10, 25 and 50 µg/ml concentrations, it was significantly stimulatory (+192.2 %, +86.6 % and +61.5 %) to the root length of *A. fatua.* At 10 µg/ml concentration, it also significantly stimulated (+80.5 %) and moderately stimulated (+50.9 %) the shoot length of *A. fatua* at 25 µg/ml concentration (Fig. 3).



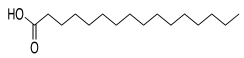
I 1- (2, 6-Dihydroxy-4-methoxy-3-methylphenyl) ethanone



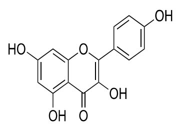
II Entiopicroside



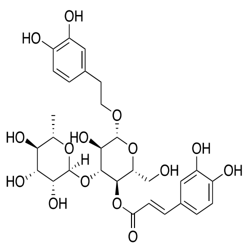
III β-Sitosterol



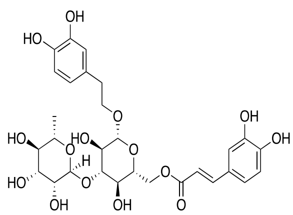
IV Palmitic acid



V Kaempferol



VI Verbascoside



VII Isoverbascoside

Figure 3. The structures of compounds I - VII isolated from potato variety Q9.

(iii). Compound III: At 50, 100, and 200 µg/ml concentrations, it was significantly stimulatory (+132.5 %, +155.0 %, and +160.4 %) to the root length of *A. fatua.* It significantly stimulated (+72.6 % and +97.0 %) the shoot length of *A. fatua* at 100 and 200 µg/ml concentrations. It was moderately stimulatory (+56.6 %) to the shoot length at 50 µg/ml concentration. It significantly stimulated (+82.1 %) at 200 µg/ml concentration and moderately (+53.6 %) at 100 µg/ml concentration to the dry weight of *A. fatua* (Fig. 3).

(iv). Compound IV: At 10, 25, 100, and 200 µg/ml concentrations, it was significantly stimulatory (+113.7 %, +82.1 %, +147.7 % and +222.7 %) to the root length of *A. fatua.* It also significantly stimulated (+68.6 %, +65.7 %, 59.3 % and +69.1 %) at 10, 25, 100 and 200 µg/ml concentrations, respectively (Fig. 3).

(v). Compound V: At 10 and 25 µg/ml concentrations, it was significantly stimulatory (+132.6 % and +73.6%) to the root length of *A. fatua.* It significantly stimulated (+92.6 %, +71.4 %, and +62.0 %) the shoot length of *A. fatua* at 10 and 25 µg/ml concentrations (Fig. 2).

(vi). Compound VI: At 10, 25, 50, 100, and 200 µg/ml concentrations, it was significantly stimulatory (+238.5 %, +228.5 %, +181.1 %, +182.5 % and +296.7 %) to the root length of *A. fatua*. It also, significantly stimulated (+135.6 %, +116.5 %, +72.3 %, +69.9 %, and +143.7 %) the shoot length of *A. fatua* at 10, 25, 50, 100 and 200 µg/ml concentrations (Fig. 3).

(vii). Compound VII: It was significantly stimulatory (+239.1 %, +109.6 %, +89.1 %, and +63.5 %) to the root length of *A. fatua* at 10, 25, 50, and 100 µg/ml concentrations. It also significantly stimulated (+84.0 %, +78.8 % and +72.6 %) the shoot length of *A. fatua* at 10, 25 and 50 µg/ml concentrations (Fig. 3).

(II). *Brassica campestris*

(i). Seeds germination: None of the isolated compounds significantly inhibited the seed germination of *B. Campestris* (Fig. 3).

(ii). Seedlings growth: The inhibitory effects of compounds **I-VII** isolated from potato var. Q9, were evaluated at 10, 25, 50, 100 and 200 µg/ml concentrations on root length, shoot length and dry weight of *B. Campestris* (Fig. 2). All compounds had inhibitory effect (Fig. 3).

Inhibitory effects on *B. campestris*: However, these compounds were not stimulatory but were inhibitory to *B. campestris* (Fig. 3).The inhibitory activity of all compounds on seedling growth was concentration-dependent (Fig. 3).

(i). Compound I: It was significantly inhibitory (-72.6 %, and -82.2 %) to the root length of *B. campestris* at 100 and 200 µg/ml concentrations. At 50 µg/ml concentration, it had only moderate inhibitory effects (-51.3 %) on the root length of *B. campestris*. It significantly inhibited (-69.6 %) the shoot length of *B. campestris* at 200 µg/ml concentration, but moderately inhibited (-52.4 %) at 100 µg/ml concentration (Fig. 3).

(ii). Compound II: It had significant inhibitory effects (-64.2 %, and -69.7 %)on the root length of *B. campestris* at 100 and 200 µg/ml concentrations, respectively. The concentrations of 25 and 50 µg/ml were moderately inhibitory (-51.8%, and -55.5%). It was moderately inhibitory (-50.0 %, -53.0 %, and -54.5 %) to shoot length of *B. campestris* at 50, 100, and 200 µg/ml concentrations, respectively (Fig. 3).

(iii). Compound III: It had moderate inhibitory effects (-51.8, -58.0 and -59.9 %) on the root length of *B. campestris* at 50, 100 and 200 µg/ml concentrations, respectively. It had no inhibitory effects on the shoot length of *B. campestris* (Fig. 3).

(iv). Compound IV: It also had moderate inhibitory effects (-57.2 % and -58.1 %), on the root length of *B. campestris* at 100 and 200 µg/ml concentrations, respectively. It moderately inhibited (-50.2 %) the shoot length of *B. campestris* at 200 µg/ml concentration (Fig. 3).

(v). Compound V: It had no significant inhibitory effects on test weed species (Fig. 3).

(vi). Compound VI: It significantly inhibited (-64.8 %) the root length of *B. campestris* at 200 µg/ml concentration, while its 50 and100 µg/ml concentrations were moderately inhibitory (-54.0%, and -55.1%). It was not inhibitory to shoot length of *B. campestris* (Fig. 3).

(vii). Compound VII: It significantly inhibited the root length (-60.5 %) of *B. campestris* at 200 µg/ml concentration. It had moderate inhibitory effects (52.7 %, 54.5 % and 58.1

%) at 25, 50 and100 µg/ml concentrations, respectively. It was also inhibitory to the shoot length of *B. campestris* (Fig. 3).

All compounds present in potato variety Q9 were stimulatory to seedlings growth (root length, shoot length, dry weight) of *A. fatua* weed. However, these compounds were inhibitory to seedlings growth of *B. campestris.* The compounds **I**, **II**, **VI** and **VII** were the main potent compounds in potato var. Q9. In fact, Compounds **VI** and **VII** were similar compounds but had different configurations.

In this study, although the inhibitory effects of compounds isolated from the Q9 variety were not higher than crude extracts, but the potent compounds **I**, **II**, **VI** and **VII** were more inhibitory. Maybe the allelopathic effects were due to the synergistic reactions between the different compounds or their fractions. Thus, in future studies, we will identify new and effective isolated compounds with significant inhibitory effects on the *B. campestris* weed and find the allelopathic mechanisms of methanolic extracts of Q9 variety.

Delay in seed germination affects the ability of the seedlings to establish themselves in natural conditions (3). An indirect relation exists between lower germination rate and allelochemicals, as the reduced germination might be the consequence of restricted uptake of water (21) or alteration in the synthesis/activity of gibberellic acid (15). The allelochemicals reduces the plant growth (roots and shoots length) and biomass (12,23). The seedling growth was more sensitive to allelochemicals than the seed germination (5). Root membranes are main spot of action for phenolic acids. Allelochemicals also cause lignification of roots resulting in reduced growth (6). Allelochemicals inhibits the elongation, expansion and division of cells (8) which is a pre-requisite for seedling growth. Reduced shoot length might be due to the inhibitory effects of allelochemicals on minerals uptake and their translocation.

CONCLUSIONS

The variety Q9 was chosen as allelopathic variety, because its aqueous extracts were more inhibitory than X65 variety. From the methanoilc extracts of potato variety Q9,7-compounds [1- (2, 6-Dihydroxy-4-methoxy-3-methylphenyl) ethanone (**I**), Gentiopicroside (**II**), β-sitosterol (**III**), palmitic acid (**IV**), kaempferol (**V**), verbascoside (**VI**) and isoverbascoside (**VII**)] were isolated. All compounds present in potato variety Q9 stimulated the seedlings growth (root length, shoot length, dry weight) of *A. fatua* weed, but inhibited the seedlings growth of *B. campestris* weed. The compounds **I**, **II**, **VI**, and **VII** were the main active compounds, these inhibited the seedling growth of *B. campestris* but were stimulatory to *A. fatua*.

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REFERENCES

1. Abdelgaleil, S.A.M. and Hashinaga, F. (2007). Allelopathic potential of two sesquiterpene lactones from *Magnolia grandiflora* L. *Biochemical Systematics and Ecology* **35:** 737-742.

2. Beare-Rogers, J., Dieffenbacher, A. and Holm, J.V. (2001). Lexicon of lipid nutrition (IUPAC Technical Report). *Pure and Applied Chemistry* **73**: 685-744.
3. Chaves, N., Sosa, T. and Escudero, J.C. (2001). Plant growth inhibiting flavonoids in exudates of *Citrus ladanifer* and in associated soils. *Journal of Chemical Ecology***27**: 623-631.
4. Chen, B.M., Peng, S.L. and Ni, G.Y. (2009). Effects of the invasive plant *Mikania micrantha* H.B.K. on soil nitrogen availability through allelopathy in South China. *Biological Invasions* **11**: 1291-1299.
5. Chung, I.M. and Miller, D.A. (1995). Allelopathy influence of nine-forage grass extracts on germination and seedling growth of alfalfa. *Agronomy Journal* **87**: 767-772.
6. Dos Santosh, W.D., Ferrarese, M.L.L., Finger, A., Teixeira, A.C.N. and Ferrarese-Filho, O. (2004). Lignifications and related enzymes in *Glycine max* root growth-inhibition by ferulic acid. *Journal of Chemical Ecology* **30**: 1199-1208.
7. Duke, S.O. (2007). Weeding with allelochemicals and allelopathy-a commentary. *Pest Management Science* **63**: 307-307.
8. Einhellig, F.A. (1995). Mechanism of action of allelochemicals in allelopathy. In: *Allelopathy: Organisms, Processes, and Applications* (Eds. Inderjit, K.M.M. Dakshini and F.A. Einhellig) pp. 96-116 American Chemical Society, Washington, DC.
9. Faizi, S., Siddiqui, B.S., Saleem, R., Saddiqui, S. and Aftab, K. (1994). Isolation and structure elucidation of new nitrile and mustard oil glycosides from *Moringa oleifera* and their effects on blood pressure. *Journal of Natural Products* **57**: 1256-1261.
10. Guo, L.Z., Guo, Q.Y., Xin, C.Y., Wei, Y.H. and Weng H. (2007). Research in experiment of 96% S-metolachlor for controlling weeds in potato fields. *Plant Protection* **6**: 54-56. (Chinese)
11. Hadacek, F. (2002). Secondary metabolites as plant traits: current assessment and future perspectives. *Critical Review of Plant Science* **21:** 273-322.
12. Hussain, M.I., González, L. and Reigosa, M.J. (2010). Phytotoxic effect of allelochemicals and herbicides on photosynthesis, growth and carbon isotope discrimination in *Lactuca sativa*. *Allelopathy Journal* **26**: 157-174
13. Lobo-Echeverri, T., Rivero-Cruz, J., Su, B.N., Chai, H.B., Cordell, G.A., Pezzuto, J.M., Swanson, S.M., Soejarto, D.D. and Kinhorn, A.D. (2005). Constituents of the leaves and twigs of *Calyptranthes pallens* collected from an experimental plot in southern Florida. *Journal of Natural Products* **68**: 577-580.
14. Miyase, T., Koizumi, A., Ueno, A., Noro, T., Kuroyanagi, M., Fukushima, S., Akiyama, Y. and Takemoto, T. (1982). Studies on the Acyl Glycosides from *Leucoseptrum japonicum*. *Chemical and Pharmaceutical Bulletin* **30**: 2732-2737.
15. Olofsdotter, M. (1998). Allelopathy in rice. In : *Proceeding of the workshop on allelopathy in rice*. (Ed Olofsdotter, M.) Manila, Philippines, International Rice Research Institute. 25-27 Nov. 1996.
16. Osbourn, A.E., Qi, X., Townsend, B. and Qin, B. (2003). Dissecting plant secondary metabolism - constitutive chemical defences in cereals. *New Phytologist* **159**: 101-108.
17. Porte, A.J., Lamarque, L.J., Lortie, C.J., Michalet, R. and Delzon, S. (2011). Invasive *Acer negundo* outperforms native species in non-limiting resource environments due to its higher phenotypic plasticity. *BMC Ecol*ogy **11**: 28
18. Ricciardi, A., 2004. Assessing species invasions as a cause of extinction. *Trends in Ecology & Evolution* **19**: 619-619.
19. Rudkowska, I., Abu Mweis, S.S., Nicolle, C. and Jones, P.J. (2008). Cholesterol-lowering efficacy of plant sterols in low-fat yogurt consumed as a snack or with a meal. *Journal of the American College of Nutrition* **27**: 588-595.
20. Takeda, Y., Masuda, T., Honda, G., Takaishi, Y., Ito, M., Aahurmetov, O.A., Khodzhimatov, O.K. and Otsuka, H. (1999). Secoiridoid Glycosides from *Gentiana olivieri*. *Chemical and Pharmaceutical Bulletin* **47**:1338-1340.
21. Tawaha, A.M. and Turk, M.A. (2003). Allelopathic effects of black mustard (*Brassica nigra*) on germination and growth of wild barley (*Hordeum spontaneum*). *Journal of Agronomy and Crop Science* **189**: 298-303.
22. Vasconsuelo, A. and Boland, R. 2007. Molecular aspects of the early stages of elicitation of secondary metabolites in plants. *Plant Science* **172**: 861-875.
23. Ye, S.F., Yu, J.Q., Peng, Y.H., Zheng, J.H. and Zou, L.Y. (2004). Incidence of *Fusarium* wilt in *Cucumis sativus* L. is promoted by cinnamic acid, an autotoxin in root exudates. *Plant and Soil* **263:** 143-150.