Detection of phytochemical compounds and pigments in seeds and shoots of *Cuscuta campestris* parasitizing on eggplant

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Abstract This study aimed to detect the chemical compounds contained in *Cuscuta campestris* seeds and shoot parts using the GC–MS following methanol extraction method, and also determining protein, total phenolic, total soluble carbohydrates, plant hormones and pigments for both seeds and shoot (flower and filament). The GC–MS analysis results showed six compounds are common between seed and shoot and the presence of different compounds in different plant parts including 24 compounds in seeds and 9 compounds in the shoot parts. The content of protein and carbohydrate in shoot (1.095% and 22.640 mg g⁻¹ respectively) were higher than in seeds and the total phenols content in seeds (11.094 mg g⁻¹) was greatest than in shoot. In addition, there were differences between seeds and filaments parts in terms of concentration of pigments and free plant hormones, filaments were superior in total chlorophyll and carotene content (5.37 mg 100 g⁻¹ and 13.26 mg 100 g⁻¹ respectively). In seeds, ABA content was very high reaching 93.38 µM compared to other hormones, which had an effect on the seed dormancy of *C. campestris*.

Keywords *C. campestris* · Eggplant · GC–MS · Seeds · Shoot · Filaments

Introduction

Dodder (*C. campestris*) is a species of weed (parasitic plant) of the family Convolvulaceae and it has many common names (dodder, beggar vine, strangle weed, field dodder and golden dodder). *C. campestris* is widespread worldwide due to expansion of vegetative reproduction (Shen et al. 2013), in addition to the production of large quantities of seeds with low weight (Holm et al. 1997). Crops that are infested by *C. campestris* include: eggplants, potatoes, alfalfa, clover, fababean, tomatoes, beets, carrots and to lesser extent asparagus, chickpeas, grapevine, melon, onions and some ornamental plants (Dawson et al. 1994; Kroschel 2001).

In Iraq, eggplant (*Solanum melongena*) is considered as a most common plant of Solanaceae, which is affected by *C. campestris*, due to the lack of knowledge and the absence of selective herbicide to control *C. campestris* without damaging host plant (eggplant).

The difficulty of *C. campestris* control is represented in the vegetative reproduction and long dormancy of hard seed up to 50 years or more (Costea and Tardif 2004). *C. campestris* (dodder) suck all of the nutrients, photosynthetic metabolite, nitrogen, macro/micro minerals, water, phytohormones and other active compounds from their host plant (Nickrent and Musselman 2004; Shen et al. 2013; Smith et al. 2013) and reduce the carboxylation efficiency and CO₂ saturated rate of photosynthesis. Dodder contains photosynthetic pigments (chlorophyll α , b and other accessory pigments) and nutritional representation varied from Hetro or Hemiparasitism to Holoparasitism

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(Braukmann et al. 2013). Also, dodder contains many chemical compounds (coumarin, alkaloids, flavonoids, α -amarin, β -amarin, caffeic acid, ascorbic acid, oleonic acid, stigmaterol, glycoside, Isohamentin 3-O neohesperidoside and sitisterol) that is used in local manmade medicine (Srivastava 2002; Hassan et al. 2014), which are dietary nutritional supplements to enhance human health and prevention of oxidative-related diseases (Yena et al. 2008). Different extraction methods were used to detect active compounds (polar groups) in plant using GC–MS, but methanol extraction was preferred for revealing many phytochemical compounds (Jayapriya and Gricilda 2015). Also, these substances differed qualitatively and quantitatively according to the species of host plant (Bais and Kakkar 2014). Moreover, anti-inflammatory effect was confirmed in *C. campestris* (Agha et al. 1996). While, Movsesyan and Azaryan (1974) reported that ingestion of *C. campestris* by animals resulted in toxicity and was if it exceeds 5% of the total roughage.

The current study aims to identify the chemical structure differences in *C. campestris* between shoot and seeds and to determine the plant pigments and phytohormones in *C. campestris* and its relation to seed dormancy. In addition to the systematic significance, this study also provides a foundation for future studies on the physiology and biochemistry of *C. campestris*.

Materials and methods

C. campestris material

The fresh *C. campestris* samples were collected from infected eggplant in Najaf, at the complete growth-stage (flowers and seeds) (Bais and Kakkar 2014) and washed individually under running tap water to remove any traces of dirt. and molecular diagnosis of *C. campestris* was recorded as a new strain at the National Center for Biotechnology Information (NCBI) under the new accession number (MG669312). The seeds were purified and then were isolated from dodder shoot (flowers with filaments). The two samples were dried at 70 °C for 2 days in an oven. They were then macerated to powder form with a mixer grinder (Hassan et al. 2014).

C. campestris preparation for GC–MS analyzes

The method of Sen and Batra (2012) was used as follows: about 10 g of the dodder seeds and dodder shoot powdered samples were soaked in 100 ml methanol individually. Samples were left for 24 h so as to dissolve alkaloids, flavonoids and other constituents. The methanol extract

was filtered using Whatman No. 1 filter paper and the residue was discarded.

GC–MS analysis

Qualitative analysis, the samples of both seeds and shoot methanol extraction were performed by GC–MS with a Hewlett–PackardG1800A GCD system comprising an HP-5971 gas chromatograph with electron ionization detection. Compounds were separated on an HP-5MS (Agi-lent Technologies) ultra-low-bleed 5% phenyl polydimethylsiloxane capillary column (30 m $\times$ 0.25 mm i.d. $\times$ 0.25 μ M film thickness). Splitless injection was performed with a purge time of 1.0 min. The carrier gas was helium at a flow rate of 1 ml min^{−1}. The column temperature was maintained at 40 °C for 4.0 min, then programmed at 5° min^{−1} to 80 °C and then at 10 min^{−1} to 280 °C. The inlet temperature was 250 °C, the detector temperature 280 °C and the solvent delay 4 min. The two samples were analyzed at same method.

Compound identification

The molecular formula was used for the identification of compounds in NIST/EPA/NIH and Wiley 7th Mass Spectral Libraries and comparison with published spectral data were used for identification of the compounds. The compounds were identified by National Center for Biotechnology Information, U.S. National Library of Medicine, 8600 Rockville Pike, Bethesda and MD20894.

Determination of % protein

Protein percentage was calculated using Kjeldahl method according to AOAC (2005).

Determination of total phenolic compound (mg g^{−1})

Total phenol analysis was performed using Folin–Ciocalteu reagent, described in Abrun et al. (2016) according to Slinkard and Singleton (1997). The quantification was made from calibration curve using Gallic acid as standard and calculation were performed by equation of the linear regression obtained from calibration curve by UV–Visible spectrophotometer.

Determination of total soluble carbohydrates (mg g^{−1})

Total soluble carbohydrates was determined according to phenol–sulphuric acid method (Dubois et al. 1956). The

quantification was made from calibration curve using Glucose as standard and calculation were performed by equation of the linear regression obtained from calibration curve by UV–Visible spectrophotometer.

Analytical procedure of pigments

Seeds and filaments of *C. campestris* content of pigments were analyzed, according to Goodwin (1976) and Sumanta et al. (2014) by UV–Visible spectrophotometer.

Determination of free hormones (μM)

Seeds and filaments of *C. campestris* content of free plant hormones were determined according to Ergun et al. (2002). The quantification was made from calibration curve using IAA, GA_3 , ABA and Zeatin as standard and calculation performed by equation of the linear regression obtained from calibration curve by UV–Visible spectrophotometer.

Results

GC–MS analysis

The current study discriminated and differentiated the chemical compounds present in the flower and filaments of *C. campestris* from those present in the seeds. There are

many differences in the chemical compounds between the seeds and the shoot (flower and filament) although with the presence of six similar compounds in both parts. Unlike dodder plant body, its seeds contain many medicinal compounds (antibiotics, antimicrobials) that are beneficial to human health and other substances that are considered as dietary supplement for humans as well as containing other compounds useful in industry (Tables 1, 2, Figs. 1, 2).

Protein, total phenols and total soluble carbohydrates content of seeds and shoot of *C. campestris*

Estimated the concentration of protein and total soluble carbohydrates in *C. campestris* seeds (0.681% and 13.390 mg g^{-1} respectively) were lower than in shoot (1.095% and 22.640 mg g^{-1} respectively). While, the total phenols content in seeds was 11.094 mg g^{-1} compared to 8.137 mg g^{-1} in shoot of (Table 3, Figs. 3, 4).

Pigments content of seeds and filaments of *C. campestris*

Estimated the content of chlorophyll a and b and carotene in *C. campestris* seeds and filaments (Table 4). The chlorophyll content in the seeds ($a = 1.65 \text{ mg } 100 \text{ g}^{-1}$ and $b = 1.23 \text{ mg } 100 \text{ g}^{-1}$) was lower than in the filaments ($a = 2.96 \text{ mg } 100 \text{ g}^{-1}$ and $b = 2.41 \text{ mg } 100 \text{ g}^{-1}$). The

Table 1 Results of GC–MS analysis of flower samples and filaments (shoot) of *C. campestris*, the compound highlighted is common and present in both the shoot and the seed

Peak	Retention time (min)	Name of compound	Molecular formula	Molecular weight (g mol^{-1})	Peak area (%)
1	3.023	Nickel tetracarbonyl	$\text{Ni}(\text{CO})_4$	170.73	4.67
2	7.319	Maltol	$\text{C}_6\text{H}_6\text{O}_3$	26.11	3.94
3	13.775	Trifluoroacetic acid, n-tridecyl ester	$\text{C}_{15}\text{H}_{27}\text{F}_3\text{O}_2$	296.36	2.11
4	14.176	(1R,3R,4R,5R)-(–)-Quinic acid	$\text{C}_7\text{H}_{12}\text{O}_6$	192.16	10.38
5	17.840	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$\text{C}_{36}\text{H}_{60}\text{O}_6$	176.12	17.60
6	19.834	9,12-Octadecadienoic acid (Z,Z)	$\text{C}_{18}\text{H}_{32}\text{O}_2$	280.45	5.91
7	19.898	cis,cis,cis-7,10,13-Hexadecatrienal	$\text{C}_{16}\text{H}_{26}\text{O}$	234.38	10.81
8	23.259	1-Heptacosanol	$\text{C}_{27}\text{H}_{56}\text{O}$	396.73	7.51
9	23.436	Hexadecanoic acid, 2-hydroxy-1 (hydroxymethyl)ethyl ester	$\text{C}_{19}\text{H}_{38}\text{O}_4$	330.50	4.88
10	26.356	Hexatriacontane	$\text{C}_{36}\text{H}_{74}$	506.98	5.47
11	27.382	gamma.-Tocopherol	$\text{C}_{28}\text{H}_{48}\text{O}_2$	416.69	2.02
12	27.893	alpha.-Tocopherol-beta.-D-mannoside	$\text{C}_{35}\text{H}_{60}\text{O}_7$	592.85	4.69
13	28.583	Ergost-5-en-3-ol (3.beta.)	$\text{C}_{28}\text{H}_{48}\text{O}$	400.69	3.85
14	28.772	Stigmasterol	$\text{C}_{29}\text{H}_{48}\text{O}$	412.70	3.69
15	29.254	gamma.-Sitosterol	$\text{C}_{29}\text{H}_{50}\text{O}$	414.71	12.47
					100.00

Table 2 Results of GC–MS analysis of seeds sample of *C. campestris*, the compound highlighted is common and present in both the shoot and the seeds

Peak	Retention time (min)	Name of compound	Molecular formula	Molecular weight (g/mol)	Peak area (%)
1	11.261	Benzofuran, 2,3-dihydro-	C ₈ H ₈ O	120.15	12.53
2	11.920	2H-Pyran-2-one, tetrahydro-6-propyl-	C ₈ H ₁₄ O ₂	142.19	2.36
3	12.221	2-Methoxy-4-vinylphenol	C ₉ H ₁₀ O ₂	150.17	4.26
4	13.526	Benzofuran-2 carboxaldehyde	C ₉ H ₆ O ₂	146.14	10.43
5	13.999	D-Allose	C ₆ H ₁₂ O ₆	180.15	3.06
6	14.875	3-Deoxy-d-mannonic lactone	C ₆ H ₁₀ O ₅	162.14	3.36
7	15.152	1,2,3,5-Cyclohexanetetrol, (1.alpha., 2.beta., 3.alpha., 5.beta.)	C ₆ H ₁₂ O ₄	148.15	5.44
8	17.251	2-Pentadecanone, 6,10,14-trimethyl	C ₁₈ H ₃₆ O	268.48	0.44
9	18.261	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.43	5.34
10	19.233	1-Octadecanol	C ₁₈ H ₃₈ O	270.50	2.18
11	19.577	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280.45	3.11
12	19.617	Trifluoroacetic acid,n-tridecyl ester	C ₁₅ H ₂₇ F ₃ O ₂	296.36	3.16
13	19.777	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284.48	1.81
14	20.640	2-Piperidin-1-yl-6,7-dihydro-oxazolo[3,2-a][1,3,5]triazin-4-one	C ₁₀ H ₁₄ N ₄ O ₂	222.24	6.96
15	20.708	6H-Indolo[3,2,1-de][1,5]naphthyridin-6-one	C ₁₄ H ₈ N ₂ O	220.23	0.93
16	20.795	7,8-Dihydro-9H-pyrido[1,2,3-cd]perimidin-9-one	C ₁₄ H ₁₀ N ₂ O	222.24	0.88
17	20.960	n-Tetracosanol-1	C ₂₄ H ₅₀ O	354.66	0.61
18	21.879	n-Tetracosanol-1	C ₂₄ H ₅₀ O	354.66	0.61
19	22.008	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C ₁₉ H ₃₈ O ₄	330.50	2.18
20	22.110	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	C ₁₆ H ₂₂ O ₄	278.34	0.63
21	23.006	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	C ₂₁ H ₄₀ O ₄	356.54	4.50
22	23.146	Octadecanoic acid, 2,3-dihydroxypropyl ester	C ₂₁ H ₄₂ O ₄	358.56	0.96
23	23.498	Indolo[2,3-a]quinolizine, 1,2,3,4,6,7,12,12b-octahydro-	C ₁₈ H ₂₄ N ₂	268.40	2.83
24	24.422	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-	C ₂₇ H ₄₆ O ₂	402.66	2.88
25	25.128	gamma.-Tocopherol	C ₂₈ H ₄₈ O ₂	416.69	3.75
26	25.717	alpha.-Tocopherol-beta.-D-mannoside	C ₃₅ H ₆₀ O ₇	592.85	0.51
27	26.275	2,6-Bis(3,4-methylenedioxyphenyl)-3,7-dioxabicyclo(3.3.0)octane	C ₂₀ H ₁₈ O ₆	354.35	2.40
28	26.727	Campesterol	C ₂₈ H ₄₈ O	400.69	3.25
29	26.964	Stigmasterol	C ₂₉ H ₄₈ O	412.70	1.43
30	27.601	gamma.-Sitosterol	C ₂₉ H ₅₀ O	414.71	7.21
					100.00

carotene content in the filaments was 13.26 mg 100g⁻¹ compared to 2.40 mg 100 g⁻¹ in the seeds.

Free hormones (μM) in seeds and filaments of *C. campestris*

Determination of plant hormones content in *C. campestris* is shown in (Table 5, Figs. 5, 6, 7, 8). In seeds, ABA content was very high reaching 93.38 μM compared to other hormones, especially GA₃, which was 0.39 μM, 0.21 μM for IAA and 1.07 μM for Zeatin. The ABA

content in the filaments was also relatively high (14.11 μM) than other hormones which was higher than those in seeds reaching 4.03 μM, 2.98 μM and 0.28 μM for GA₃, Zeatin and IAA, respectively.

Discussion

The chemical analysis of the active compounds in the dodder plant, especially its seeds, reflects the level of benefits and damage to host (Yena et al. 2008; Biswas et al.

Fig. 1 The GC–MS analysis of shoot samples of *C. campestris*

2012; Hassan et al. 2014). In *C. campestris* (MG6–69,312) (Tables 1, 2), the six compounds common to seeds and shoot seems to be essential and inherited and are not affected by host species. We believe that these compounds play important roles in increasing immunity level against adverse environmental and biotic factors. These compounds might be considered and used as vitamins, food additives and anti-cancer in humans. The differences in the chemical compounds between the seeds and the shoot of *C. campestris* explain that the shoot compounds change as the host plant changes, these changes occur mostly due to the

absorption of many mineral elements and amino acids during parasitism and this is in agreement with findings by Koch et al. (2004) and Bais and Kakkar (2014). In addition, the increase in carbohydrate and protein in shoot compared to seeds, due to *C. campestris* during the parasitization had no selective absorption of a specific compound from the host, but the absorption of many compounds necessary for growth and duration (Anis et al. 1999; Biswas et al. 2012). The increase in total phenols in *C. campestris* seeds in comparison with dodder shoot may occur for two reasons; the first reason is the phenolic patterns prove to be stable in

Fig. 2 The GC–MS analysis of seed samples of *C. campestris*

Table 3 Protein, total phenols and total carbohydrates content in the seeds and shoot of *C. campestris*

<i>C. campestris</i>	% Protein	Total phenols (mg g ⁻¹)	Total soluble carbohydrates (mg g ⁻¹)
Shoot	1.095	8.137	22.640
Seeds	0.681	11.094	13.390

both dodder species irrespective of different host plants (Löffler et al. 1995), the second reason is that there are many different types of phenolic compounds in seeds and this is in agreement with findings by Hongzhu and Jiashi (1997). Because of the composition of seeds in the final stage of physiological growth of *C. campestris*, current

study suggest that the effective compounds obtained from the seeds can be used to differentiate between *Cuscuta* species and this might be added to the classification keys of this plant. As shown in Table 4, chlorophyll content in *C. campestris* varies according to species. As mentioned previously, dodder body content is affected by the amount

Fig. 3 Standard curve of gallic acid

Fig. 4 Standard curve of glucose

of substances and dyes contained in the host tissues, which is found in this study and is in consistent with Dinelli et al. (1993). The presence of pigments of chlorophyll a, b and carotene confirms diverse abilities in photosynthetic activities (Braukmann et al. 2013). Therefore, *Cuscuta* spp. can be classified under parasitic weeds and cannot be limited to the group of plant pathogens as commonly adopted in Iraq especially among those interested in plant diseases. The dark color of seeds, pigments and differences in phytohormones, especially the increase concentration of ABA and reduction of GA₃ (Tables 4, 5), all are factors which may have some role in preventing water absorption and increasing dormancy of *C. campestris* seeds, agreeing with Bewley (1997), Zhang et al. (2008) and Büyükkartal et al. (2013). The ABA's superiority over other plant hormones in the filaments of *C. campestris* may be

Table 5 The estimation of free plant hormones in the seeds and filaments of *C. campestris*

<i>C. campestris</i>	IAA μ M	GA ₃ μ M	ABA μ M	Zeatin μ M
Seeds	0.21	0.39	93.38	1.07
Filaments	0.28	4.03	14.11	2.98

Fig. 5 Standard curve of IAA

 Fig. 6 Standard curve of GA₃

Fig. 7 Standard curve of ABA

Table 4 Pigments content in the seeds and filaments of *C. campestris*

<i>C. campestris</i>	Chlorophyll a (mg 100 g ⁻¹)	Chlorophyll b (mg 100 g ⁻¹)	Total chlorophyll (mg 100 g ⁻¹)	Carotene (mg 100 g ⁻¹)
Seeds	1.65	1.23	2.80	2.40
Filament	2.96	2.41	5.37	13.26

Fig. 8 Standard curve of zeatin

explained by accumulative or multiple genes expression in order to maintain dormancy in the resulting seeds (Li and Foley 1997; Garelo et al. 2000). Also the high concentration of growth-promoting hormones (IAA, GA₃ and Zeatin) in filament of *C. campestris* lead to increased vegetative growth and its spread on host plant.

Conclusions

There were differences between seeds and filaments parts in terms of concentration of pigments and free plant hormones. Filaments were superior in total chlorophyll and carotene content. According to GC–MS and UV–Visible spectrophotometer determination of chemical compounds in shoot and seeds of dodder (*C. campestris*), similarity of some of these compounds content may reflex the absorbed and the translocation of these compounds from the host plant. Presence of such compounds and phenols in seeds may prolong the dormancy. Chlorophylls and carotene content of filaments may have an impact on the growth of dodder plant before finding the host plant, in particular the presence of promoting plant hormones (IAA, GA₃ and Zeatin) at high concentration. Seeds content of ABA (growth inhibitor hormone) may be explained on the basis of delaying germination and increase in seed dormancy period.

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