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Full Length Article

Allelopathic Role of Essential Oils in Sunflower Stubble on Germination and Seedling Growth of the Subsequent Crop

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Abstract

The phytotoxic effect of sunflower extracts prepared by different plant parts on germination and seedling growth of potentially crop rotational plants like wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), lentil (*Lens culinaris* Medik.) and sunflower (*Helianthus annuus* L.) were studied. Stem and head extracts of sunflower cv. Sanbro at 2.5, 5 and 10% concentrations were obtained from sunflower residuals after harvest. Germination percentage, mean germination time, root and shoot length, seedling fresh and dry weight were investigated. Also, electrical conductivity (EC) and pH values were measured at each concentration and essential oil compounds of both stem and head extracts were determined. Results showed that germination and seedling growth of the species were significantly influenced by sunflower extract doses. Barley appeared to be more tolerant to phytotoxicity of sunflower, while sunflower was the most susceptible. Higher doses of extract inhibited germination and seedling growth of the investigated plants. EC values of both extracts were raised by increasing level of extracts without significantly changing pH. The main essential oil compound of head extract was α-pinene with 54.70% followed by Calarene with 10.82% while Benzofuran (13.65%), palmitic acid (13.52%) and trans-verbenol (11.89%) were mostly found in stem extract. There were apparently differences between stem and head extracts of sunflower in terms of toxicity and stem extract had less inhibitory effect on germination and seedling growth of target plants than head. It was concluded that the inhibitory effect of head extract was mainly due to higher EC values and α-pinene in essential oil composition and removing the head of sunflower should be beneficial for alleviating the allelopathic effect.

Keywords: *Helianthus annuus*; Phytotoxicity; Phenolics; pH; EC

Introduction

Vegetable oil production in Turkey has mainly depended on sunflower, which is cultivated in arid and semi arid regions due to its high adaptation ability, low labor needs and suitable for mechanization (Ozer *et al.*, 2004; Kazemeini *et al.*, 2009). These advantages of sunflower make it more important in crop rotation under rainfed conditions where only few crops produce acceptable yield. Consequently, crop sequence gains importance for increasing productivity of crop plants under dry conditions.

The crops cultivated after sunflower may be adversely influenced due to toxic residues, low nutrients and moisture in soil and high pest populations after sunflower (Robinson, 1978). It was found that sunflower residues in soil had inhibitory effects on sunflower and many other species (Schon and Einhellig, 1982; Mehboob *et al.*, 2000; Naseem *et al.*, 2009). The reason of the inhibitory effect has been expressed as allelopathic substances in sunflower residues (Farooq *et al.*, 2011). Bogatek *et al.* (2006) stated that the inhibitory effect of sunflower leaf aqueous extracts harvested at the beginning of flowering was determined in mustard seed germination and seedling growth. Nikneshan *et al.* (2011) determined that there were differences in allelopathic effects of sunflower cultivars and allelopathic potential could be used in weed management in safflower and wheat. Moreover, several researchers indicated that phytotoxic effects of sunflower were useful for natural weed control (Leather, 1983; Ashrafi *et al.*, 2008; Alsaadawi *et al.*, 2011; Miri, 2011)

Most of the researches on allelopathic effects of sunflower have been concentrated on weed control using fresh plant materials collected before harvest. The aim of the present study was to focus on the reason of sunflower phytotoxic effects on crops in rotation and inquiring differentiation in allelopathic substances in relation to plant parts by using sunflower stubble after harvest.

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Materials and Methods

Sunflower cultivar Sanbro MR, which is extensively grown in Turkey, was used as test plant. Sunflower was grown under rainfed conditions of Ankara-TURKEY in 2010 and stubbles were collected after harvest. Plants were separated into stem and head parts. Plant parts were ground to fine powder after air-dried. Stem and head powder of 25, 50 and 100 g were soaked in 1 L of distilled water at 25°C for 24 h. After soaking, each solution was filtered through two layers of filter paper and the filtrate centrifuged at 7500 rpm for 5 min. Consequently, stem and head extract doses were arranged as 0 (distilled water), 2.5, 5 and 10%. Distilled water served as a control. Electrical conductivity (EC) and pH of each solution were measured using WTW 3.15i conductivity meter and pH meter.

Seeds of barley cv. Aydanhanım, wheat cv. Tosunbey, lentil cv. Meyveci-2001 and sunflower cv. Sanbro MR were germinated in three rolled filter papers with 10 mL of respective test solutions with concentrations of 2.5, 5 and 10%. The rolled paper with seeds was put into sealed plastic bags to avoid moisture loss. Seeds were allowed to germinate at 20±1°C for barley and wheat, and 25±1°C for lentil and sunflower in the dark for 10 days (ISTA, 2003). Germination was considered to have occurred when the radicles were 2 mm long. Germination percentage was recorded every 24 h for 10 days. Speed of germination was evaluated using mean germination time (MGT) as described by ISTA (2003). Root and shoot lengths and seedling fresh and dry weights were measured after the 10 days incubation.

Essential oil was obtained by hydrodistillation of stem and head samples was analyzed by GC-MS. Hewlett Packard 6890 N GC equipped with HP-5 MS capillary column (30 m × 0.25 μm) and HP 5973 mass selective detector was used for essential oil composition. Carrier gas was helium at a flow rate of 1 mL/min. Injector and MS transfer line temperatures were set at 220 and 290ºC, respectively. Column temperature was initially kept at 50ºC for 3 min then gradually increased to 150ºC at a 3ºC/min rate, held for 10 min and finally raised to 250ºC at 10ºC/min. Diluted samples (1/100 in acetone, v/v) of 1.0 μL were injected automatically and in the splitless mode. Individual components were identified by spectrometric analyses using computer library.

A randomized complete block design was used with a factorial arrangement of treatments (extract source and extract dose) with 4 replications and 50 seeds in each replicate. Data were analyzed by two way analysis of variance using the statistical package MSTAT-C, and the differences among the means were compared using Duncan's multiple range test ($p<0.05$).

Results

Increasing extract doses led to significantly decreased seed germination of barley ($p<0.05$). Similar germination percentage for head and stem extracts were observed (Table 1). Germination percentage declined from 99.5% to 97.0% regardless of extract source. MGT was delayed by increasing extract dose. Stem extract influenced it more severely than did head. Increasing head and stem extract doses resulted in 3.63 and 1.57 d longer duration, respectively. Root length was affected by extract doses and it was reduced considerably at 2.5% of extract. Shoot length decreased in response to increasing dose of extract, while this decrease was more prominent in head extract. Compared to stem, head extract was more detrimental to shoot growth. Each increase in extract dose caused a remarkable decrease in seedling fresh weight. Higher extract dose resulted in higher seedling dry weight in barley.

Extract doses slightly affected germination percentage of wheat and negligible variation was determined (Table 2). On the other hand, MGT was delayed when extract dose increased. Wheat seeds exposed to head extract germinated faster compared to seeds in stem extract. Germination was extended by increasing extract dose with 2.68 d in head and 1.19 d in stem. Root length was shortened by increasing extract doses, while the differences between stem and head were insignificant. Only head extract adversely affected shoot length but, it was enhanced by stem extract. Seedling fresh weight showed similarity with variation in shoot length. However, seedling dry weight was not significantly changed by extract sources and doses.

The detrimental effect of extract dose on germination of lentil appeared at 10% extract concentration (Table 3). However, MGT was retarded by increasing extract dose. Apparent significant difference was recorded between extract sources and stem extract had a more retarder effect on germination than that of head. Head extract had the minimum time to germinate under all the extract doses. Increasing extract drastically shortened root length in lentil. The reduction in root length was more prominent in seedlings exposed to stem extract. Each increase in extract dose reduced in shoot length and the shortest shoot was obtained from dose of 10%. Head extract seriously reduced seedling fresh weight of lentil. The lowest fresh weight was measured at 10% of head extract. Higher extract dose caused reduction in seedling dry weight. Seedling dry weight was more severely affected by head extract compared to stem extract.

As shown in Table 4, germination percentage of sunflower was dramatically reduced by increasing stem extract dose, while head extract caused a slightly significant decrease. Furthermore, time to germination was seriously delayed by stem extract; shorter germination time was observed at head extract. Sunflower seeds germinated rapidly in control with 1.26 d, while increased extract doses resulted in delayed MGT. Contrarily, lower root length was measured at increased head extract doses, especially at 5% and above. Shoot length declined when extract doses increased, reaching minimal (1.06 mg plant^{-1}) at head extract dose of 10%. The deleterious effect of head extract

Table 1: Influence of sunflower stem and head extracts on germination and seedling growth of barley

Extract source	Extract dose (%)				Mean
	Control	2.5	5	10	
Germination percentage (%)					
Stem	99.5±1.0	99.0±1.2	98.0±1.6	97.0±2.6	98.4
Head	99.5±1.0	99.0±1.2	97.5±1.0	97.0±1.2	98.3
Mean	99.5^{a}	99.0^{ab}	97.8^{bc}	97.0^{c*}	
Mean germination time (day)					
Stem	1.31^{f}±0.13	2.06^{e}±0.12	2.69^{c}±0.15	4.94^{a}±0.04	2.75
Head	1.31^{f}±0.13	2.23^{d}±0.09	2.32^{d}±0.12	2.88^{b}±0.08	2.18
Mean	1.31	2.14	2.50	3.91	
Root length (cm)					
Stem	4.66±0.97	3.84±0.40	2.61±0.10	2.18±0.16	3.32
Head	4.66±0.97	3.49±0.59	3.04±0.14	2.60±0.14	3.45
Mean	4.66^{a}	3.67^{b}	2.82^{c}	2.39^{c}	
Shoot length (cm)					
Stem	13.61^{a}±0.94	13.98^{a}±0.94	9.88^{b}±0.65	7.69^{c}±0.52	11.29
Head	13.61^{a}±0.94	9.28^{b}±0.61	7.23^{c}±1.51	3.87^{d}±0.29	8.38
Mean	13.61	11.64	8.55	5.54	
Seedling fresh weight (mg plant^{-1})					
Stem	255^{a}±9.5	268^{a}±13.6	225^{b}±9.29	191^{d}±9.70	235
Head	255^{a}±9.5	218^{bc}±12.6	206^{c}±11.23	139^{e}±3.30	205
Mean	255	243	216	165	
Seedling dry weight (mg plant^{-1})					
Stem	42.5±2.47	49.0±3.76	50.9±2.81	54.1±1.58	49.1
Head	42.5±2.47	47.0±2.62	46.3±1.92	48.4±1.04	46.1
Mean	42.5^{c}	48.0^{b}	48.6^{b}	51.2^{a}	

Table 2: Influence of sunflower stem and head extracts on germination and seedling growth of wheat

Extract source	Extract dose (%)				Mean
	Control	2.5	5	10	
Germination percentage (%)					
Stem	100±0.0	100±0.0	99.5±1.0	99.5±1.0	99.8
Head	100±0.0	100±0.0	98.5±1.0	98.5±1.0	99.3
Mean	100^{a}	100^{a}	99.0^{b}	99.0^{b*}	
Mean germination time (day)					
Stem	1.33^{d}±0.06	2.10^{c}±0.21	2.50^{b}±0.11	4.01^{a}±0.09	2.48
Head	1.33^{d}±0.06	2.12^{c}±0.03	2.19^{c}±0.09	2.52^{b}±0.10	2.04
Mean	1.33	2.11	2.35	3.26	
Root length (cm)					
Stem	5.28±0.40	4.29±0.47	3.90±0.40	2.10±0.16	3.89
Head	5.28±0.40	4.16±0.59	3.00±0.28	2.08±0.29	3.63
Mean	5.28^{a}	4.22^{b}	3.45^{c}	2.09^{d}	
Shoot length (cm)					
Stem	7.78^{c}±0.60	11.82^{a}±0.31	11.77^{a}±0.68	7.80^{c}±0.08	9.79
Head	7.78^{c}±0.60	10.24^{b}±0.36	7.40^{c}±0.30	3.30^{d}±0.14	7.18
Mean	7.78	11.03	9.59	5.55	
Seedling fresh weight (mg plant^{-1})					
Stem	153^{b}±13.6	167^{a}±12.2	168^{a}±3.78	139^{bc}±4.43	157
Head	153^{b}±13.6	153^{b}±7.36	131^{c}±4.78	89^{d}±24.9	132
Mean	153	160	149	114	
Seedling dry weight (mg plant^{-1})					
Stem	29.0±0.62	29.6±2.22	31.7±0.76	34.1±2.42	31.1
Head	29.0±0.62	30.4±1.55	31.4±1.89	30.5±7.81	29.7
Mean	29.0	30.0	31.6	32.3	

Data represent mean±standard deviation (SD) of four replicates, *: Means with the same letter(s) are not significantly different at p<0.05 level

was apparent on shoot growth. Similarly, seedling fresh weight was adversely influenced by increasing extract dose especially prepared by head. No significant difference was noted in seedling dry weight and any clear trend was not observed.

Table 3: Influence of sunflower stem and head extracts on germination and seedling growth of lentil

Extract source	Extract dose (%)				Mean
	Control	2.5	5	10	
Germination percentage (%)					
Stem	97.0±3.83	99.0±1.2	98.0±1.6	84.8±4.9	94.7
Head	97.0±3.83	94.5±3.8	92.5±3.4	89.5±6.8	93.4
Mean	97.0^{a}	96.8^{a}	95.3^{a}	87.1^{b*}	
Mean germination time (day)					
Stem	1.87^{e}±0.16	2.36^{d}±0.14	2.87^{b}±0.09	5.21^{a}±0.15	3.08
Head	1.87^{e}±0.16	2.35^{d}±0.09	2.51^{cd}±0.06	2.61^{c}±0.06	2.33
Mean	1.87	2.35	2.69	3.91	
Root length (cm)					
Stem	2.78^{a}±0.13	1.78^{c}±0.07	1.50^{d}±0.25	1.17^{e}±0.16	1.81
Head	2.78^{a}±0.13	2.43^{b}±0.10	1.85^{c}±0.07	1.30^{de}±0.14	2.09
Mean	2.78	2.11	1.68	1.23	
Shoot length (cm)					
Stem	5.22±0.44	4.15±0.48	2.83±0.61	1.71±0.26	3.48
Head	5.22±0.44	3.97±0.34	2.63±0.38	1.55±0.30	3.34
Mean	5.22^{a}	4.06^{b}	2.73^{c}	1.63^{d}	
Seedling fresh weight (mg plant^{-1})					
Stem	85.3^{a}±9.21	89.3^{a}±6.60	67.3^{b}±5.44	48.3^{c}±6.65	72.5
Head	85.3^{a}±9.21	82.5^{a}±8.40	60.8^{b}±4.79	19.8^{d}±2.06	62.1
Mean	85.3	85.9	64.0	34.0	
Seedling dry weight (mg plant^{-1})					
Stem	9.13±0.61	8.65±0.65	7.00±0.41	5.58±0.43	7.59^{a}
Head	9.13±0.61	8.05±0.71	6.28±0.40	4.33±0.24	6.94^{b}
Mean	9.13^{a}	8.35^{b}	6.64^{c}	4.95^{d}	

Table 4: Influence of sunflower stem and head extracts on germination and seedling growth of sunflower

Extract source	Extract dose (%)				Mean
	Control	2.5	5	10	
Germination percentage (%)					
Stem	96.0^{a}±0.0	92.5^{ab}±5.74	86.0^{b}±9.1	37.0^{c*}±12.4	77.9
Head	96.0^{a}±0.0	98.0^{a}±1.6	96.0^{a}±4.0	96.5^{a}±1.9	96.6
Mean	96.0	95.3	91.0	66.8	
Mean germination time (day)					
Stem	1.26^{f}±0.09	2.61^{c}±0.07	3.23^{b}±0.14	6.12^{a}±0.24	3.30
Head	1.26^{f}±0.09	2.01^{e}±0.03	2.11^{de}±0.11	2.32^{d}±0.10	1.90
Mean	1.26	2.31	2.67	4.17	
Root length (cm)					
Stem	5.37±0.99	6.15±0.91	1.87±0.16	1.72±0.09	3.78^{a}
Head	5.37±0.99	4.89±0.62	1.85±0.27	1.00±0.19	3.28^{b}
Mean	5.37^{a}	5.52^{a}	1.86^{b}	1.36^{b}	
Shoot length (cm)					
Stem	6.99^{a}±0.56	4.61^{b}±0.30	2.89^{c}±0.74	2.13^{d}±0.16	4.16
Head	6.99^{a}±0.56	3.31^{c}±0.52	1.80^{d}±0.24	1.06^{e}±0.05	3.29
Mean	6.99	3.96	2.35	1.59	
Seedling fresh weight (mg plant^{-1})					
Stem	479^{a}±32.9	423^{b}±55.2	296^{cd}±30.5	267de±16.3	366
Head	479^{a}±32.9	329^{c}±19.2	233^{e}±11.4	171^{f}±15.4	303
Mean	479	376	265	219	
Seedling dry weight (mg plant^{-1})					
Stem	69.8±3.04	61.4±9.22	71.1±1.36	71.6±1.50	68.5
Head	69.8±3.04	67.6±2.31	64.9±4.23	66.9±6.98	67.3
Mean	69.8	64.5±	68.0	69.3	

Data represent mean ± standard deviation (SD) of four replicates, *: Means with the same letter(s) are not significantly different at p<0.05 level

Electrical conductivity and pH values of head and stem extract doses were illustrated in Figure. Particularly a remarkable increase in EC values was determined by increasing extract dose. Higher EC values at the same concentration were obtained from head extract. pH did

not significantly vary with extract doses, while pH of head extract reduced slightly when extract dose increased.

As shown in Table 5, essential oil sunflower revealed that the presence of 15 compounds in head extract, and predominant one was α-Pinene (54.70%) followed by Calarene (10.82%) and Kaurene (6.39%). Stem extract contained 20 compounds from total essential oil content of 93.59%. Four main compounds detected were Bezofuran (13.65%), palmitic acid (13.52%), trans-verbenol (11.89%) and Kaurene (10.88%).

Discussion

Both sunflower extracts adversely affected germination and subsequent growth parameters of wheat, barley, lentil and sunflower. Average reduction in germination percentage was 29.8% in sunflower, 9.9% in lentil, 2.5% in barley and 1.0% in wheat, while stem extract was more hazardous on germination compared to head extract (Table 4). Ashrafi *et al.* (2003) found that seed germination of wild barley and sunflower was reduced by sunflower extract, especially whole plant extract. Our results were confirmed by Mehboob *et al.* (2000) in linseed and Bogatek *et al.* (2006) in mustard, who affirmed that inhibitory effect of sunflower extracts on seed germination was observed. Moreover, the present study revealed that both sunflower extracts had no toxic effect on germination of wheat and barley, because all seeds germinated under all doses of both extracts. On the other hand, the vital effect of sunflower extracts was the retarding of germination rather than inhibiting germination. Seeds of tested plants exposed to stem extract needed to longer time to germinate than head extract (Table 1–4). This may result from differences in essential oil composition, that is, α-pinene usually founded in the oil of several coniferous trees especially the pine and phytotoxic effect on germination (Abrahim *et al.*, 2000) was the major compound in head extract, while several compounds in stem extract were detected. Alsaadawi *et al.* (2011) argued that sunflower genotypes with higher phenolic compounds appeared to be more phytotoxic to weeds and wheat than the other genotypes.

Greater reduction in root length was well evident at 10% ($p<0.05$) with similar reduction in both stem and head extracts (Table 2 and 3). The most affected roots were detected in sunflower and extract dose of 5% reduced root length. However, shoot length was dramatically decreased when head extract concentration was increased. Depending on decrease in shoot growth, seedling fresh weight gradually declined with increasing sunflower head extract. Increasing sunflower extract doses did not result in a remarkable decrease in seedling dry weight. Although tested plants showed different responses to each of the applied extracts and changed dry weight to varying degrees, sunflower extract enhanced seedling dry weight in barley and wheat. The reason of limited seedling growth of the species might

Table 5: Essential oil composition of sunflower stem and head extracts

Compounds	RT	%	
		Head	Stem
α-pinene	10.05	54.70	3.60
Verbenene	10.75	2.83	-
Sabinene	11.59	1.07	-
β-pinene	11.71	1.06	-
p-cymene	13.89	1.17	-
α-campholene aldehyde	18.70	2.52	2.67
trans-pinocarveol	19.24	-	4.02
cis-verbenol	19.38	-	2.95
trans-verbenol	19.58	-	11.89
Myrtenol	21.96	-	2.23
trans-Carveol	23.01	-	1.27
Borneol	26.09	1.55	1.24
Calarene	32.45	10.82	9.35
β-bisabolene	35.52	3.57	-
Calarene epoxide	37.54	-	1.68
Spathulenol	38.24	1.67	1.51
Caryophyllene oxide	38.41	1.50	1.52
Dehydroaromadendrene	38.99	-	1.51
Isolongifolene	40.36	1.13	5.47
β-himachalene	40.49	2.64	2.65
Benzofuran	42.47	4.56	13.65
2-pentadecanone	43.63	-	0.69
Cyclohexadecane	44.10	-	1.29
Palmitic acid	45.16	-	13.52
Kaurene	48.13	6.39	10.88
Total %		97.18	93.59
Essential oil content %		0.12	0.04

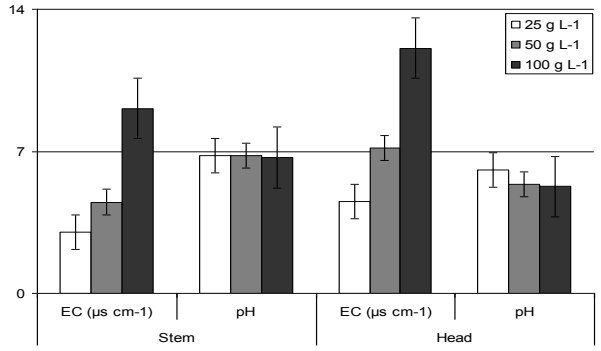

Fig. 1: Changes in electrical conductivity (EC) and pH values of the extract doses prepared by using sunflower stem and head. Letters on each bar show significance level of means at $p<0.05$ level

be an increase in EC and decrease in pH with increased extract doses. These results were similar to those observed by Alsadaawi *et al.* (2011) in wheat, Bogatek *et al.* (2006) in mustard, Mehboob *et al.* (2000) in linseed, Schon and Einhellig (1982) in sorghum. Also, Batish *et al.* (2002) stated that higher EC values were found in the field after sunflower harvest. However, our results showed that essential oil compounds might have a potential role on germination of the investigated plants (Batish *et al.*, 2002; Alsaadawi *et al.*, 2011; Farooq *et al.*, 2011).

In conclusion, average harvest index of sunflower under rainfed conditions of Turkey was approximately 45% and biomass production of sunflower was 5000 kg ha^{-1}. The amount of stubble equals to 1.76 kg ha^{-1} of head and 0.32 kg ha^{-1} of stem essential oil. Essential oil in stem and head extracts was more effective to have allelopathic effect of sunflower rather than EC and pH values, which might have secondary effect. If the cultivation of sunflower in dry areas is required, a crop plant like barley and wheat should be preferred. Moreover, sunflower may not be a subsequent crop and sunflower stubble head should be removed before cultivating next crop.

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