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RESEARCH ARTICLE

Chemical Composition and Phytotoxicity of Chickpea (*C. arietinum* L.)

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Abstract

The aqueous and organic extracts of *Cicer arietinum L.* were assayed at different concentrations to assess their phytotoxicity. The extracts were tested on *Setaria verticillata*, a weed species. The final germination percentages and the seedling shoot and root lengths were reduced by aqueous extracts of *C. arietinum*. The reduction in germination and seedling growth was more at higher concentrations. Also, the organic extracts inhibited the germination and seedling growth of target species. The ethyl acetate fraction was the most toxic and the highest concentration (6mg/mL) was the most active. Quantitative and qualitative analysis of *C. arietinum* aerial parts ethyl acetate extract was carried out by gas chromatography-mass spectrometry (GC-MS). A total of 59 compounds were identified in the extract, among them many are known by their herbicidal effect. These results encourage optimizing the use of this biomass which might be helpful to develop new and potent natural herbicides.

Keywords: *Cicer arietinum; Setaria verticillata;* Extracts; Chemical Composition; Phytotoxicity; Germination; Growth

Introduction

The widespread use of herbicides has resulted in the increasing incidence of weeds' resistance, in environmental pollution and in associated health problems [1]. Therefore, there is need for alternative weed management strategies which are less synthetic herbicide dependent or based on naturally occurring compounds [2]. Allelopathy is a natural technique that may be considered as a tool for biological weed control and in crop production [3,4]. Allelopathy has been defined as the inhibitory or stimulatory effects of a plant or microorganism on other plants through the release of chemical compounds into the environment [5]. Allelopathic chemicals are released into the environment by four ecological processes: volatilization, leaching, decomposition of plant residues in soil and root exudation [6]. The search and development of new herbicides through the identification of active compounds from allelopathic plants is an interesting research area [7]. These compounds can be regarded as 'natural herbicides'. Several higher plants are reported by the present authors to possess allelopathic potential and efforts are being made to apply them for weed control [8,9].

Allelopathy has been investigated in some beans such as in *P. sativum* [10], *Mucuna pruriens* [11] and *Cicer arietinum* L. [12]. It has been found that *P. sativum* shoots contain pisatin, which has been identified as an inhibitory chemical [10]. The objectives of the present study were to evaluate the phytotoxicity of *Cicer arietinum* by testing their aqueous and organic extracts on *Setaria verticillata* weed. Quantitative and qualitative analysis of the ethyl acetate extract obtained from the aerial parts of chickpea was done by GC-MS.

Materials and Methods

Plant materials

Aerial parts of chickpea (*C. arietinum*) were harvested in the stage of maturity. Plants were washed with tap water and then oven-dried at 60 °C for 72 h, thereafter they were ground to obtain a fine powder stored until needed.

Extraction

Aqueous extracts: Dried plant material (120g) was soaked in 1.0L distilled water at room temperature for 24 h. The extract was filtered several times using cheesecloth and kept at 4 °C in the dark until use.

Organic extracts: Sequential extraction was done with organic solvent with increasing polarity: petroleum ether, ethyl acetate and methanol. Samples of 100g dried powdered was extracted, successively in organic solvents for 7 days at room temperature. The organic extracts were evaporated to dryness under reduced pressure at 45-50 °C, using a Rotavapor R-114 (Buchi, France). The residue was weighed to determine yield. The dried fractions were stored at 4 °C until use. The extracts were tested at three concentrations (2, 4 and 6 mg/mL) in bioassays.

Bioassays

Tests with aqueous extracts: Aqueous extract of *C. arietinum* was diluted with distilled water to give final concentrations of 15, 30, 50 and 120gL^{-1}. They were tested on *Setaria verticillata,* a weed species. Seeds were surface-sterilized with 0.525g/L of sodium hypochlorite for 15 min, and then rinsed four times with deionized water. Twenty imbibed seeds of target species were separately placed on the filter paper in sterilized Petri dishes (9cm); 5 mL of respective extracts were applied per Petri dish as per treatments. The seeds were irrigated with distilled water in control. The Petri plates were then placed in a growth chamber [25±2 °C temperature, 16/8 h light / dark period, and 75% relative humidity]. Treatments were arranged in completely randomized design with 4-replications. Germinated seeds were counted daily till 7 days [13]. Data were transformed to percent of control for analysis.

The germination index (GI) was determined as under [14]:

$GI = (N1)^{*} 1 + (N2\text{-}N1)^{*} 1/2 + (N3\text{-}N2)^{*} 1/3 + ... + (Nn\text{-}Nn\text{-}1)^{*} 1/n$

Where, N1, N2, N3…, Nn: proportion of germinated seeds observed afterwards 1, 2, 3…, n-1, n days. This index shows the germination delay induced by the extract [15].

For growth tests, twenty pre-germinated seeds, with 1mm root length, of target species were separately placed on the filter paper in 9 cm diameter Petri dishes and 5mL of each extract were applied as per treatment. Seedlings irrigated with distilled water served as control. Shoot and root length of receiver species were measured 7- days after sowing. Data were transformed to percent of control for analysis.

The inhibition or stimulation (%) was calculated as under [16]:

Inhibition (-)/stimulation (+) % = [(extract – control)/control] ×100

Where extract is the parameter measured in presence of *C. arietinum* extract, and control is the parameter measured in presence of distilled water.

Tests with organic extracts: For organic extracts studies, to determine the chemical group to which the active molecules of the extracts of chickpea residues could belong, residues concentrated from petroleum ether, ethyl acetate and methanol were dissolved in methanol to prepare 3-concentrations (2, 4 and 6 mg/ ml) to estimate their effects on germination and early growth of target species. There were two controls: distilled water and MeOH, to eliminate the effects of organic solvents. The effect of organic extracts on seed germination and seedling growth was determined as previously described.

Gas chromatography-Mass spectrometry (GC-MS) analyses

Extraction with organic solvent: Powdered *C. arietinum* aerial parts (2g) was extracted with 10ml of ethyl acetate at rate of 20% (w/v) [17] in the dark for 24h in a shaker (Eyela Model MMS-300, Tokyo Rikakikai Co., Ltd., Japan) at room temperature and then the solvent was evaporated by vacuum rotary evaporator (EYELA N1000, Japan). After filtration (0.45µm), extract obtained was then transferred to vials and kept in the dark at -20 °C prior to use [18], in order to prevent changes in the chemical components.

Gas chromatography-Mass spectrometry (GC-MS) analyses

Quantitative and qualitative analysis of the extract were carried out by gas chromatography -mass spectrometry (GC-MS). GC-MS was performed in a Hewlett-Packard 5972 MSD System. An HP-5 MS capillary column (30 m x 0.25 mm ID, film thickness of 0.25 µm) was directly coupled to the mass spectrometry. The carrier gas was helium, with a flow rate of 1.2 ml/min. Oven temperature was programmed (50 °C for 1 min, then 50-250 °C at 5 °C/min) and subsequently held isothermal for 4 min. Injector port: 250 °C, detector: 280 °C, split ratio: 1:50. Volume injected: 1µl of 5% solution (diluted in hexane); mass spectrometer: HP5972 recording at 70 eV; scan time: 1.5 s; mass range: 50-500 amu (atomic mass unit). Software adopted to handle mass spectra and chromatograms was ChemStation.

The identification of the compounds was based on mass spectra (compared with Wiley 275.L, 6th edition mass spectral library). Further confirmation was done from Retention Index data generated from a series of alkanes retention indices (relatives to C6-C28 on the HP-5 MS column) [19-24].

Statistical analysis

The laboratory bioassays were done in complete randomized design with four replications. Data were subjected to ANOVA and Duncan tests (p=0.05) with IBM SPSS Statistics version 20), for Windows program, to analyze treatment differences.

Results

Aqueous extract effects on germination and growth

For germination, the results showed that *C. arietinum* aqueous extract significantly affected the seed germination of *S. verticillata* (Table 1). Aqueous extracts of chickpea induced total inhibition of the weed germination at 120 g/L. The seed germination (%) was reduced by increasing concentration levels. Thus, the lowest germination index was recorded at 50 g/L (GI= 34.17%). Inhibition of germination (G) and germination index (GI) was concentration dependent. The IC50 (concentration of extract inducing 50% reduction of germination) value was 30g/L and the MIC (concentration of extract inducing 100% reduction of germination) value of extract was 120 g/L.

Aqueous extracts / concentration (gL^{-1})	GI (% of control)	% Germination
Control	-	68.75^{d}
15	78.56^{c}	61.25^{cd}
30	57.11^{c}	48.75^{bc}
50	34.17^{b}	35^{b}
120	0^{a}	0^{a}

Means with the same letters in a column are not significantly different at $P < 0.05$

Table 1: Germination index (GI), expressed in % of control, and germination percentage (%) of *Setaria verticillata* in presence of aqueous extracts of *C. arietinum* aerial parts at different concentrations

For seedling growth, the aqueous extract of *C. arietinum* was toxic at all concentrations, especially for roots which were more sensitive than shoots. Seedling growth was inhibited at the lowest concentration (15 g/L) (Figure 1). At the highest concentration (120 g/L) *C. arietinum* aqueous extract reduced the root growth of *S. verticillata* by 84%. For the shoot growth, at 30 g/L and 50 g/L a slight stimulating effect was observed with extract by 6% and 7% respectively. The inhibition of shoot growth was 17% at higher concentration (120 g/L).

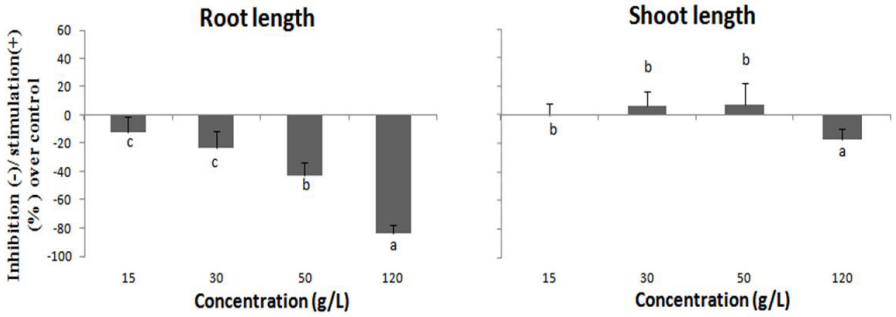

Figure 1: Inhibition (-)/stimulation (+) (% of control) of root and shoot length of *Setaria verticillata*, 7 days after pre-germination, in presence of aerial parts aqueous extracts of *Cicer arietinum* on root and shoot length of *Setaria verticillata*. The bars on each column show standard error. Values (N=4±S.E.). Different letters in columns indicate significant differences among treatments at P<0.05 (LSD test)

Organic extract effects on germination and growth

To determine the chemical group to which bioactive molecules of *C. arietinum* extract could be owned three organic extracts (petroleum ether, ethyl acetate and methanol) were tested. Organic residues were dissolved in methanol, which required a methanol control. Results showed that this solvent did not affect germination of *S. verticillata*, hence effects could be attributed to allelochemicals present in organic extracts. The ethyl acetate extract was the most toxic to weed species, followed by methanol, and then petroleum ether extracts (Table 2). In addition the highest concentration was the most active. Thus, at 6 mg/mL, extracts of *C. arietinum* gave only 2.5% germination rate, recorded with the ethyl acetate extract.

	Concentration (mg/mL)	GI (% of control)	%Germination
Extract	Control	-	81.25^{f}
Petroleum ether	2	72.10^{d}	73.75^{ef}
	4	64.88^{d}	65^{e}
	6	43.95^{c}	48.75^{d}
EthylAcetate	2	23.77^{bc}	28.75^{bc}
	4	11.47^{ab}	13.75^{ab}
	6	2.21^{a}	2.5^{a}

	Concentration (mg/mL)	GI (% of control)	%Germination
Methanol	2	36.23^{c}	38.75^{cd}
	4	12.40^{ab}	12.5^{a}
	6	10.89^{ab}	7.5^{a}

Means with the same letters in a column are not significantly different at $P < 0.05$.

Table 2: Germination index (GI), expressed in % of control, and germination percentage (%) of *S. verticillata* in presence of organic extracts of *C. arietinum* aerial parts at different concentrations

Seedling growth of *S. verticillata* varied with the origin and the concentration of organic extracts. The roots were more sensitive than the shoots. Moreover, the ethyl acetate fraction was significantly more toxic, compared to the two others. Thus, at 6mg/mL, root and shoot length revealed significant respective inhibitions of 83.59% and 34.67%, in the presence of the ethyl acetate fraction (Figure 2). Besides, growth of roots was reduced by all organic extracts, except the methanol extracts (2mg/mL) which caused stimulation by 23.66%. The petroleum ether (6mg/mL) and the methanol extracts (at 2mg/mL and 4mg/mL) caused 4.65%, 14.02% and 8.38% stimulation in *S. verticillata* shoot growth, respectively.

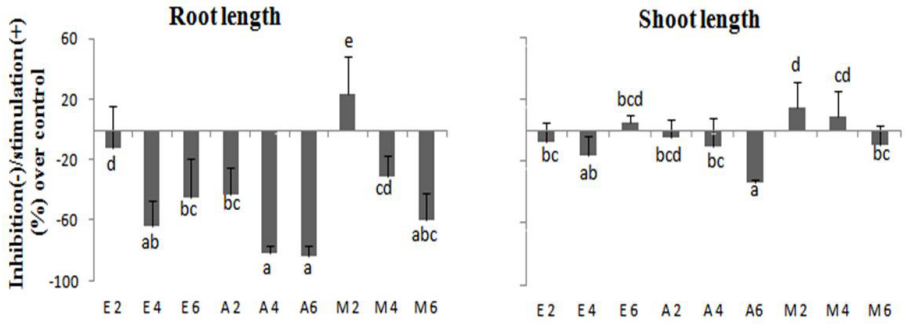

Figure 2: Inhibition (-)/stimulation (+) (% of control) of root and shoot length of *Setaria verticillata,* 7 days after pre-germination, in presence of three organic extracts of *Cicer arietinum* aerial parts: Petroleum ether (E), ethyl acetate (A) and methanol (M) (at 2,4 and 6mg/mL). The bars on each column show standard error. Values (N=4±S.E.). Different letters in columns indicate significant differences among treatments at P<0.05(LSD test)

Chemical composition of the extract

The constituents of *C. arietinum* aerial parts are listed in order of their elution on the HP-5 MS capillary column and the results are presented in Table 3.

No	Compound	IR	%
1	But-2-enal	610	0.17
2	3-Methylbutanal	625	12.11
3	2-Methylbutanal	635	1.01
4	(E)-Z-Butenal	649	1.44
5	Pentane-2, 3-dione	660	3.28
6	2-Ethylfuran	684	0.45
7	(E)-Hex-2-enal	829	0.25
8	4-Methyloctane	863	0.07
9	2-Methyloctane	864	0.09
10	Heptanal	870	0.14
11	2-Butylfuran	886	0.12
12	Heptan-2-one	890	0.07
13	γ-Butyrolactone	908	0.98
14	α-Thujene	923	0.93
15	α- Pinene	931	15.81
16	Benzaldehyde	932	0.28
17	(E) Hept-2-enal	934	0.16
18	Camphene	938	6.07
19	(E)-2-Heptenal	952	0.50
20	Octan-3-one	959	0.26
21	4-Methylnonane	962	0.17

No	Compound	IR	%
22	Oct-1-en-3-ol	966	0.26
23	β-Pinene	968	5.37
24	3-Octanol	974	7.56
25	2-Pentylfuran	979	2.16
26	α-Phellandrene	997	2.63
27	n-Decane	1000	0.23
28	α-Terpinene	1009	0.72
29	p-Cymene	1019	0.12
30	Phellandrene	1021	0.06
31	Limonene	1022	0.05
32	(E) Oct-3-enal	1033	0.23
33	(E)-β-Ocimene	1037	0.31
34	γ-Terpinene	1055	1.41
35	α-Terpinolene	1082	2.46
36	3- Methyldecane	1092	0.36
37	Farnesane	1095	0.58
38	4-Methylundecane	1160	4.11
39	3-Methylundecane	1171	5.61
40	3,8- Dimethyldecane	1172	0.12
41	4- Carvomenthenol	1178	2.88
42	α- Terpineol	1189	0.96
43	n-Dodecane	1200	0.80
44	4-Methyldodecane	1259	0.39
45	2-Methyldodecane	1264	0.27
46	3-Methyldodecane	1271	0.12
47	NonanoicacidFatty	1275	1.11
48	2,4-Decadienal Fatty	1292	3.48
49	Carvacrol	1298	3.34
50	(E,E)-2,4-Decadienal	1315	0.40
51	trans-Caryophyllene	1419	0.34
52	α-Humulene	1483	0.91
53	n-Pentadecane	1500	0.80
54	n-Hexadecane	1600	0.65
55	γ-Eudesmol	1628	0.87
56	Globulol	1633	1.14
57	T-Cadinol	1640	0.67
58	δ -Cadinol	1669	0.43
59	Hexadecanoicacid	1967	0.10
	Total identified		98.38
Grouped compounds			
	Aldehydes		16.68
	Alcohols		15.01
	Oxygenated sesquiterpenes		3.10
	Ketones		4.59
	Alkanes		14.38
	Monoterpenes		35.94
	Furans		2.74
	Fatty acids		4.69
	Sesquiterpenes		1.25

Kovats retention index (RI) relative to C6-C28 n-alkanes on the HP-5 MS capillary column

Table 3: Chemical composition of *Cicer arietinum* aerial parts revealed by GC-MS

Discussion

This study was conducted to evaluate the phytotoxic effect of aqueous and organic extracts from a food plant, such as chickpea, through testing them on a weed species (*S. verticillata*). Aqueous extracts of *C. arietinum* caused significant inhibition on germination and seedling growth. Their phytotoxicity was much stronger when concentration increased; they provoked a significant inhibition at higher concentration. The toxicity of aqueous extracts of plants on germination has been reported by Hoque*, et al.*, Siddiqui, *et al.,* Pooya, *et al*., El Ayeb, *et al.*, El-Shora and Abdd El-Gawad, Saad, *et al.* and Abdd El-Gawad, *et al.* [12,25-30]. The inhibitory effect of the tested plants extract on seed germination may be due to the presence of putative allelochemicals [31], which prevented the growth of embryo, or caused its death due to chromosomal aberrations in dividing cells [32].

For growth, the aqueous extracts of *C. arietinum* were toxic, especially for roots which were more sensitive than shoots. These results are in agreement with the results of previous worker [33,34] who documented this aspect of greater sensitivity of roots compared to shoot. Thus, similar findings have been reported earlier (Ladhari, *et al.,* Omezzine and Haouala, Saad, et al., Zribi, *et al.*) [29,35-37]. In our study, the average percentages respective inhibition of roots and shoots growth were 84% and 17% at higher concentration (Figure 1). These results agree with earlier studies reporting that water extracts of allelopathic plants had more pronounced effects on roots, rather than hypocotyls growth [38]. Furthermore, the permeability of plant tissue to allelochemicals is reported to be greater than that to shoot tissue [39,40] showed that the reduction in seedlings length may be attributed to the reduced rate of cell division and cell elongation. *S. verticillata* shoot growth appeared less sensitive to allelochemicals with 5% and 7% stimulation at 30g/L and 50g/L respectively. This phytotoxicity could be attributed to several bioactive compounds that act in a synergistic manner or to compounds which regulate one another such as flavonoid, phenolic acids, saponin, alkaloids and tannins [30]. These results are in accordance with other studies, which reported that some compounds could act as inhibitors and stimulators at the same time [41]. Stimulatory effects at low concentrations of allelochemicals may become inhibitory at higher concentration [42]. Moreover, a great number of compounds stimulatory to the growth of seedlings have been found, such as agrostemin, allantoin, and strigols [43].

Organic extracts significantly affected the seed germination. Germination was especially inhibited by the ethyl acetate extract which was the most toxic and gave only 2.5% for germination rate followed by methanol, and then petroleum ether extracts (Table 2). A similar result was registred by Omezzine, *et al.* [44] who mentioned that the ethyl acetate fraction from fenugreek at the highest concentration was the most active on lettuce germination. Hoagland and Williams [45] suggested that some concentrations of an allelochemical severely reduced the seed germination, while others only depress or delay the germination. The delay in seed germination can have important biological and ecological implications, because it can affect the ability of the seedling to establish themselves in natural conditions [46]. Inhibition of seed germination was attributed to some allelopathic compounds that interact with mitochondrial membrane [47] and was strongly correlated with the inhibition of glycolysis enzymes activities and the oxidative pentose phosphate pathway (OPPP) [48]. For seedling growth, the highest toxicity was registered with the ethyl acetate fraction. Because of their different polarities, the organic extracts contain different allelochemicals groups, which explain their differential toxicity [36]. As for the inhibition of germination, that of growth could be attributed to several bioactive compounds that act in a synergistic manner or to compounds which regulate one another such as flavonoid, phenolic acids, saponin, alkaloids and tannins [30]. Phenols allelochemicals can also lead to increased cell membrane permeability and increase lipid peroxidation followed by slow growth or death of plant tissue [40].

For chemical composition of the *C. arietinum* aerial parts, there is a marked predominance of monoterpenes with an abundance of α-pinene, camphene and β-pinene. The presence of some of these terpenes (β-pinene, α-pinene, β-cymene and limonene) has been reported in dry beans [49,50] and chickpea [51]. The presence of limonene in vacuum steam volatile oil of dry red beans has been also reported [51].

Monoterpenes such α-pinene, β-pinene, limonene and p-cymene are known to have herbicidal activity [53]. Additionally, the chickpea was also characterized by the presence of aldehydes. Lasekan, *et al.* [54] found that aldehydes were identified as some of the principal compounds of the roasted chickpea. Thus, aldehydes are known to be derived from the thermal Strecker oxidative degradation of amino acids and fatty acids [55]. The high alkanes have also been reported in dry beans [50]. Alkanes are mostly derived from oxidative decomposition of lipids [56]. Alcohols were also identified in chickpea and dry beans [50,54]. Abdelgaleil, *et al.* [57] reported that sesquiterpenes caused strong inhibitory effects. Indeed α-humulene is known for their phytotoxic effects [58].

Conclusion

It was concluded that different extracts of *C. arietinum* had greater inhibitory effect on germination rate and seedling length of *S. verticillata* weed and more inhibition was seen at the highest concentration. In addition, the ethyl acetate fraction had the strongest inhibitory effects and chemical analysis has shown that it is rich in allelochemicals known by their phytotoxicity. These results encourage optimizing the use of this biomass in a crop weed control strategy, reducing our reliance on dreaded industrial products.

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